Framework for Assessing Health Impacts of Multiple Chemicals and Other Stressors (Update)

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PREFACE

The mission of the Agency for Toxic Substances and Disease Registry (ATSDR) is *to serve the public by using the best science, taking responsive public health actions, and providing trusted health information to prevent harmful exposures and disease related to toxic substances.* The U.S. Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, also known as the Superfund act) mandates that ATSDR determine whether people living near or at a hazardous waste site are being exposed, have been exposed, or will be exposed to toxic substances, whether that exposure is harmful, and what can be done to stop or reduce harmful exposures. CERCLA requires that ATSDR consider the following factors when evaluating the possible public health impacts of communities near Superfund sites: (1) the nature and extent of contamination at a site; (2) the demographics of the site population; (3) exposure pathways that may exist at a site (the extent to which people contact site contaminants); and (4) health effects and disease-related data. In addition, ATSDR is also authorized to conduct public health assessments at storage, treatment, and disposal facilities for hazardous wastes when requested by EPA, under the 1984 amendments to the Resource Conservation and Recovery Act of 1976 (RCRA). In addition, ATSDR conducts public health assessments for toxic substances, when petitioned by concerned community members, physicians, state or federal agencies, or tribal governments.

The ATSDR *Public Health Assessment Guidance Manual* (ATSDR 2005a) describes a process to prepare public health assessments that evaluate environmental data, exposure data, health effects data and community concerns. The products of this process (public health assessments, consultations, and advisories) guide the development of public health actions or recommendations such as: (1) reducing exposures (carried out by other appropriate federal, state, or tribal agencies or principal responsible parties); (2) recommending further scientific investigations when key exposure or health effects data are missing; (3) developing health education programs within an affected community; or (4) identifying community health care needs (ATSDR 2005a).

This manual, *Framework for Assessing Health Impacts of Multiple Chemicals and Other Stressors*, is a revision of ATSDR's 2004 *Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures* (ATSDR 2004a). The revised manual serves as a supplement to the ATSDR (2005a) *Public Health Assessment Guidance Manual* by describing a recommended process to evaluate the potential public health impacts of exposures to multiple chemicals and other stressors, a frequent occurrence and concern for people living in the vicinity of sites with toxic substances. This revised "mixture" manual builds on the process described in the 2004 manual and reviews scientific research advancements since 2000 related to assessing health impacts from exposures to multiple chemicals and other stressors.

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
AEGL	Acute Exposure Guideline Level
AR	androgen receptor
ATSDR	Agency for Toxic Substances and Disease Registry
BBDR	biologically based dose-response
BINWOE	binary weight of evidence
BMD	benchmark dose
BMDL	95% confidence lower limit on the benchmark dose
BTEX	benzene, toluene, ethylbenzene, and xylene
CCRE	combined cancer risk estimate
CDD	chlorinated dibenzo-*p*-dioxin
CDF	chlorinated dibenzofuran
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CHAP	Chronic Hazard Advisory Panel
AChE	acetylcholine esterase
CHO	Chinese hamster ovary
CPSC	Consumer Product Safety Commission
CRE	cancer risk estimate
CREG	Cancer Risk Evaluation Guide
dB	decibel
DHT	alpha-dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DOE	Department of Energy
EMEG	Environmental Media Evaluation Guide
EPA	Environmental Protection Agency
ER	estrogen receptor
ERPG	Emergency Response Planning Guideline
FQPA	Food Quality Protection Act
GC	gas chromatography
HBPS	high-boiling petroleum substances
HI	hazard index
HQ	hazard quotient
IARC	International Agency for Research on Cancer
IC	inhibitory concentration
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
IUR	Inhalation Unit Risk
kg	kilogram
LOAEL	lowest-observed-adverse-effect level
MCL	Maximum Contaminant Level
mg	milligram
MOA	mode of action
MOE	margin of exposure
MOS	margin of safety
MRL	Minimal Risk Level
MS	mass spectrometry
MTBE	methyl tert butyl ether
NAAQS	National Ambient Air Quality Standards

NAS	National Academy of Science
NATA	National Air Toxic Assessment
NHANES	National Health and Nutrition Examination Survey
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
OAR	Office of Air and Radiation
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OSF	Oral Slope Factor
OSHA	Occupational Safety and Health Administration
PAC	Protective Action Criteria
PAH	polycyclic aromatic hydrocarbon
PBDE	polybrominated diphenyl ether
PBPK	physiologically based pharmacokinetic
PBPD	physiologically based pharmacodynamic
PCB	polychlorinated biphenyl
PEL	permissible exposure limit
POD	point of departure
PODI	point of departure index
ppm	parts per million
RA	risk assessment
RfC	reference concentration
RfD	reference dose
RMEG	Reference Dose Media Evaluation Guide
RPF	relative potency factor
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TEEL	Temporary Emergency Exposure Limit
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalent
TLV	threshold limit value
TPH	total petroleum hydrocarbon
TTC	threshold of toxicological concern
TTD	target-organ toxicity dose
UF	uncertainty factor
U.S.	United States
VOC	volatile organic compound
WHO	World Health Organization
WOE	weight of evidence

EXECUTIVE SUMMARY

The *Framework for Assessing the Health Impacts of Multiple Chemicals and Other Stressors* is an update to ATSDR's 2004 *Guidance Manual for Assessment of Joint Toxic Action of Chemical Mixtures* (ATSDR 2004a). The revised manual is intended to assist ATSDR environmental scientists and toxicologists in determining whether combined exposure to multiple chemicals and other stressors (e.g., noise, radiation) at sites of environmental contamination may impact public health. It serves as a basis for ATSDR Interaction Profiles, as well as for ATSDR public health assessments and consultations for mixtures of toxic substances and other stressors that may be encountered by people living in the vicinity of sites of environmental contamination.

Chapter 1 of this manual provides background information that is considered important in understanding the ATSDR approach to assessing health impacts of exposure to multiple stressors. The recommended ATSDR approach described in Chapter 2 calls for a 3-tiered approach to the evaluation of exposure and toxic effects data to determine how exposure to multiple chemicals and other stressors may impact public health in ways not anticipated by single-agent analysis. Chapter 3 discusses background issues and options for assessing health impacts from multiple chemicals and other stressors, including:

1. Discussing quantitative and qualitative approaches to determining sufficient similarity among mixtures;

2. Reviewing the science underlying default assumptions of dose addition or response addition for component-based approaches;

3. Explaining (a) the hazard index approach, (b) the toxicity-organ target modification of the hazard index approach, and (c) the weight-of-evidence schemes to evaluate evidence for additivity and interactions among binary components of chemical mixtures; and

4. Briefly discussing the state of the science to incorporate other nonchemical stressors into health assessments.

The recommended ATSDR approach in Chapter 2 is meant to supplement the ATSDR (2005a) *Public Health Assessment Guidance Manual* and is generally consistent with approaches articulated by the U.S. Environmental Protection Agency (EPA) (1986, 2000, 2003) and other national and international public health or regulatory agencies (as described in Appendix C of this manual). The approach is grounded in the law (Comprehensive Environmental Response, Compensation, and Liability Act [CERCLA] and the Food Quality Protection Act [FQPA]) with the intent of affording greater assurance of protection against adverse health effects than does the assessment of each chemical separately.

The ATSDR approach outlined in Chapter 2 emphasizes the importance of an initial problem formulation to focus a practical 3-tiered approach to integrating exposure data and epidemiologic and toxicologic data and assessing potential health impacts from combined exposure to multiple agents. Parallel assessments are conducted for noncancer effects and cancer. The conclusions from this assessment can then be taken into account, *along with* biomedical judgment, community-specific health outcome data, and community health concerns, to determine the health impacts and public health actions for a site contaminated with multiple chemicals or other stressors of concern.

During problem formulation, the ATSDR approach starts with initial scoping, planning, and data collection activities. Problem formulation leads to the focus of the health assessment and includes evaluation of site history information, environmental fate and transport data, environmental media sampling data, exposure and demographics data, and community health concerns. Other outcomes of the problem formulation step include identification of chemicals and exposure pathways of concern and collection of available health-based guidance values (e.g., ATSDR Minimal Risk Levels [MRLs]) for the site-specific mixture, a sufficiently similar mixture, defined groups of chemicals within the mixture, or individual components.

In the Tier 1 preliminary evaluation, exposure estimates based on environmental media data are compared with health guidance values for single agents and chemical mixtures of concern to identify exposure pathways and agents requiring further Tier 2 or Tier 3 evaluation. Exposure pathways of concern are those with evidence that community members have, or are likely to, come in contact with a contaminant (e.g., drinking contaminated water, breathing in contaminated air, dermally contacting contaminated soil). The initial screening comparison of site-specific exposure estimates with health guidance values for single agents and defined mixtures are: (1) the ratio of an exposure estimate to the health guidance value for noncancer health effects (the hazard quotient) and (2) the product of the exposure estimate multiplied by an EPA-derived cancer slope factor for carcinogenic agents (the cancer risk estimate). Agents with hazard quotients ≥ 0.1 or cancer risk estimates $\geq 10^{-6}$ are retained for further Tier 2 analysis. Single agents with hazard quotients <0.1 or cancer risk estimates $<10^{-6}$ (e.g., 10^{-7} or 10^{-8}) are not expected to pose health impacts individually or in combination with other agents and are typically not included in Tier 2 analysis, except in those cases when scoping, planning, and data collection activities (including community health concerns) indicate that combined exposure to multiple agents could produce adverse health outcomes.

The Tier 2 analysis starts with preliminary screening evaluation of noncancer and cancer health impacts from combined exposure to multiple agents. For combined exposure to multiple agents, ATSDR recommends the use of: (1) a hazard index approach to preliminarily evaluate the potential for noncancer effects from multiple agents of concern and (2) combined cancer risk estimates from carcinogenic agents of concern. The preliminary hazard index is a sum of hazard quotients of all known agents for site-specific exposure pathways of concern (i.e., agents with individual hazard quotients ≥ 0.1), and is based on the assumption of dose addition. The combined cancer risk estimate is a sum of cancer risk estimates of all carcinogenic agents of concern associated with a site-specific exposure pathway (i.e., agents with individual cancer risk estimates $\geq 10^{-6}$), and is based on the assumption of response addition.

Further Tier 3 analysis is recommended when: (1) results of Tier 2 analyses indicate that site-specific exposure pathways have preliminary screening level hazard indices ≥ 1 or combined cancer risk estimates are $\geq 10^{-6}$; (2) concerns are high for additive or interactive joint actions (greater than or less than additive) from multiple site-specific agents of concern; and/or (3) additional site-specific health outcome data provide evidence of health effects from combined exposure to multiple agents. Depending on the availability of data and resources, additional Tier 3 analysis can include:

- evaluating and summarizing what is known and unknown about possible greater-than-additive or less-than-additive joint actions among site-specific agents of concern;
- applying a qualitative weight-of-evidence approach to assessing joint actions of binary combinations of agents of concern;
- using developed mixture/interaction physiologically based pharmacokinetic models to determine dose-dependency of possible interactions among agents of concern;
- applying more refined and stringent applications of the hazard index and combined cancer risk estimate approaches that group agents of concern based on common toxicity targets or common adverse outcomes mediated by a common mode of action;
- applying exposure estimates from probabilistic refinement of exposure models in calculating hazard indices and combined cancer risk estimates; and
- developing hazard indices and combined cancer risk estimates for specific subpopulations that may be more susceptible to the site-specific agents of concern, especially children.

1. INTRODUCTION

1.1. OVERVIEW

Assessing the health impact of chemicals or other stressors (e.g., noise, radiation) in the environment is complicated by the reality that most toxicological testing is performed on single chemicals or physical agents, but human exposures are rarely limited to single agents. Exposures resulting from hazardous waste sites or other releases into the environment generally involve more than one toxic substance agent and other stressors (ATSDR 2005a; Carpenter et al. 2002; De Rosa et al. 1996; Hansen et al. 1998; Johnson and De Rosa 1995; MacDonell et al. 2013; Mumtaz et al. 2007, 2011). This occurrence leads to concerns that exposures to multiple chemicals or other stressors may impact public health in ways not anticipated by assessing the impacts of each agent alone.

This manual presents a recommended approach to assess potential health impacts of exposures to multiple chemicals in ATSDR public health assessments and health consultations. It is an update of, and replaces, the 2004 ATSDR *Guidance Manual for the Joint Toxic Action of Chemical Mixtures* (ATSDR 2004a). This document also provides overviews of the scientific principles and evidence guiding the recommended approach.

ATSDR's public health assessments and U.S. Environmental Protection Agency (EPA) quantitative risk assessments both address potential human health effects of environmental exposures to chemicals and other agents, but they are approached differently and used for different purposes (ATSDR 2005a). ATSDR's public health assessments consider past, current, and future exposures to chemicals of concern, evaluate toxicological or epidemiological data for chemicals or mixtures of concern, and compare epidemiological or toxicological dose-response data or public health guidance values (Minimal Risk Levels [MRLs] and EPA cancer slope factors) with population exposure estimates to arrive at indices of human health impacts. The ATSDR public health assessment process focuses closely on site-specific exposure conditions and health outcome data, and considers specific community health concerns to arrive at qualitative recommendations to reduce or prevent harmful exposures or take other public health actions (ATSDR 2005a). ATSDR (2005a) guidelines for public health assessments call for early and continued coordination and communications with community members and representatives, principal responsible parties (i.e., stakeholders), EPA, and other federal, state, and local agencies. Effective coordination and communication with all interested parties throughout the process can lead to harmonization and acceptance of recommended cleanup goals (from EPA) and public health actions. In contrast, EPA's quantitative risk assessments are used as part of investigations to determine the extent to which site

remedial action (e.g., clean up) or restricted use actions (e.g., for pesticides) are needed. EPA risk assessments consider current and potential future exposures for chemicals of concern, evaluate toxicological or epidemiological data for chemicals or mixtures of concern, and compare public health guidance values (e.g., reference doses [RfDs], reference concentrations [RfCs], and cancer slope factors) for mixtures, if available, or components of mixtures, with exposure estimates for specific populations to arrive at numerical estimates of health risk if no cleanup occurs.

This first chapter of this framework manual discusses concepts and definitions that are used in assessments of potential health impacts or risks from exposure to multiple chemicals and other stressors. Chapter 2 presents ATSDR's recommended 3-tiered approach, and Chapter 3 discusses options and issues related to assessing public health impacts from exposure to multiple chemicals and nonchemical stressors. Appendices A and B provide additional background information not covered in Chapter 3, and Appendix C describes approaches recommended by other U.S. agencies and other national and international agencies.

1.2. SOME CONCEPTS AND DEFINITIONS

With the evolvement of methods to assess health impacts or risks from exposure to multiple chemicals or other stressors, various terminologies have developed that warrant some explanation in this framework (see Tables 1 and 2 for definitions of selected chemical mixture and chemical interaction terms).

"Exposure to a chemical mixture" has traditionally been used to refer to combined environmental exposure to multiple chemicals. Such mixtures can be simple, being comprised of a relatively small number of known "components", or complex, being comprised of many chemicals, often of different chemical classes. Simple mixtures may be associated with hazardous waste sites, when the number of components of concern identified in environmental media (i.e., those components presenting exposures near to or above public health guidance values) are small. The composition of complex mixtures may not be fully characterized and can vary dependent on production conditions and time since release in the environment.

Table 1. Definitions of Chemical Mixture Terms

Mixture	Any set of multiple chemicals, regardless of source and spatial or temporal proximity that may jointly contribute to actual or potential health effects in a population.
Components	The chemicals that make up a mixture.
Simple mixture	A combination of a relatively small number of chemicals that has been identified and quantified (e.g., the components of concern for a community near a hazardous waste site may constitute a simple mixture).
Complex mixture	A complex mixture has very many chemicals, often of different chemical classes. The composition of complex mixtures may not be fully characterized and can vary dependent on production conditions and time since release in the environment. Components of complex mixtures may be generated simultaneously from a single source or process (e.g., tobacco smoke), intentionally produced as a commercial product (e.g., gasoline, jet fuels, mixtures of pesticides), or coexist in environmental media as a consequence of waste disposal operations or release of components into the environment from multiple sources.
Original mixture	Any combination of all chemicals that are released into the environment at a specific point in time and location. The composition of the original mixture can change with time and location due to differential fate and transport properties of the components.
Mixture of concern	The actual mixture being evaluated in a site-specific risk assessment; often referred to as the “whole” mixture.
Sufficiently similar mixture	Sufficiently similar mixtures are those having the same chemicals but in different proportions, or having most, but not all, chemicals in common and in similar proportions. In addition, similar mixtures and their components have similar environmental fate and transport properties, and produce similar health effects, whereas dissimilar mixtures do not.
Chemical class	A group of chemicals that are similar in chemical structure and in eliciting similar biochemical sequences of events related to toxic effects, and which frequently occur together in the environment, usually because they are generated by the same process, such as manufacturing or combustion (e.g., PCBs, CDDs, PAHs).
Components of concern	The chemicals in a mixture that are likely contributors to health hazard either because their individual exposure levels approach or exceed health guidelines, or because joint toxic action with other components, including additivity or interactions, may pose a health hazard.
Index chemical	The chemical selected as the basis for standardization of toxicity of components in a group of chemicals or agents (e.g., TCDD for the assessment of dioxin-like compounds; benzo[a]pyrene for the assessment of carcinogenic PAHs).
Indicator chemical(s)	A chemical (or chemicals) selected to represent the toxicity of a mixture because it is characteristic of other components in the mixture and has adequate dose-response data (e.g., benzene has been suggested as an indicator chemical for a specific fraction of gasoline).
Aggregate exposure	The combined exposure of a population to a specific agent or stressor via multiple relevant routes, pathways, and sources.
Aggregate risk	The risk resulting from aggregate exposure to a single agent or stressor.
Cumulative risk	Cumulative risk is the combined risks from aggregate exposures to multiple agents or stressors. Cumulative risk assessment is an analysis, characterization, and possible quantification of the combined risks to health from multiple agents or stressors.

Sources: EPA 1986, 1988, 2000, 2003; Fay and Feron 1996; Hertzberg et al. 1999

CDD = chlorinated dibenzo-*p*-dioxin; PAH = polycyclic aromatic hydrocarbon; PCB = polychlorinated biphenyl; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Table 2. Interactions/Mixtures Terminology[a,b]

Interaction	When the effect of a mixture is different from the expectation of additivity based on the dose-response relationships of the individual components. In this context, additivity as "no-interaction" is the null hypothesis.
Additivity	When the effect of the mixture can be estimated from the sum of the exposure levels (weighted for potency in dose or concentration additivity) or the probabilities of effect (response additivity) of the individual components. In dose additivity (also called concentration additivity), each chemical behaves as a dilution of every other chemical in the mixture. Most stringently, each chemical contributes to the production of a common adverse outcome via a common mechanism of action. Less stringently (for screening level assessments), each chemical contributes to the production of a common adverse outcome regardless of mechanism of action. In response additivity (also called independent action), components of a mixture act independently of each other and probabilities of response to components are added.
No apparent influence	When a component that is not toxic to a particular biological system does not influence the toxicity of a second component on that system.
Synergism	When the effect of a mixture is greater than that estimated by additivity. Synergism is defined in the context of the definition of no interaction, which is usually dose additivity or response additivity. The use of "greater-than-additive" is preferred over the use of the term synergism.
Potentiation	When a component that is not toxic to a particular biological system increases the effect of a second chemical on that system.
Antagonism	When the effect of a mixture is less than that estimated by additivity. Antagonism is defined in the context of the definition of no interaction, which is usually dose additivity or response additivity. The use of "less-than-additive" is preferred over the use of the term antagonism.
Inhibition	When a component that does not have a toxic effect on a particular biological system decreases the apparent effect of a second chemical on that organ system.
Masking	When the components produce opposite or functionally competing effects on the same biological system, and diminish the effects of each other, or one overrides the effect of the other.

[a]Where effect is incidence or measured response, and additivity commonly is dose or response additivity.
[b]Based on definitions in EPA (1988, 2000, 2003), Hertzberg et al. (1999), Hertzberg and MacDonell (2002), and Mumtaz and Hertzberg (1993).

Components of complex mixtures may be generated simultaneously from a single source or process (e.g., tobacco smoke, coke oven emissions, diesel engine emissions), intentionally produced as a commercial product (e.g., gasoline, jet fuels, transformer coolants containing mixtures of polychlorinated biphenyls [PCBs]), or coexist in environmental media as a consequence of waste disposal operations or release of components into the environment from multiple sources.

Other terms related to aggregate and cumulative risk assessment have increased in frequency of use within the past 10–15 years (EPA 2003; see Table 1). This manual presents ATSDR framework for assessing health impacts from combined exposure to multiple chemicals and nonchemical stressors; the ATSDR assessment process fits within the category of cumulative risk assessment.

Assessments of health impacts or risks of simple or complex mixtures can be based on exposure data and epidemiologic or toxicologic data for the original mixture. However, following release to the environment, *simple or complex mixtures can change with time and distance from the original release site, due to the differential fate and transport of their components.* For example, immediately following a release of gasoline to soil, inhalation exposure to the more volatile components, especially the low molecular weight alkanes, may be a concern. Contamination of groundwater and surface water with the more soluble components (such as benzene, ethylbenzene, toluene, and xylene) may occur over a period of weeks to years, possibly impacting drinking water. The less mobile constituents, such as aliphatic or aromatic hydrocarbons with ≥16 carbons, may tend to remain in the soil at the site of the original release for extended periods. Thus, people living near the site of release to the environment are likely to be exposed to subsets of the original chemicals at different proportions than in the complete original mixture, and chemical composition may continue to change over time. Health guidance values based on toxicological or epidemiological data for the original mixture released into the environment may not be applicable to the actual exposures experienced by people living in the vicinity of the release.

One concern for ATSDR in terms of public health is that joint toxic action or interactions among components of a mixture of concern may increase the health hazard impact above what would be expected from an assessment of each component singly. A particular issue is whether a mixture of components, each of which is present at less than guidance concentrations, may be hazardous due to additivity, interactions, or both.

As mentioned above, toxicological interactions can either increase or decrease the apparent toxicity of a mixture relative to that expected on the basis of dose-response relationships for the components of the

mixture. Table 2 provides definitions of terms used in describing the results of interactions studies. These are the definitions that will be used in this document; other definitions exist. Some of the terms, such as dose additivity or response additivity, refer to the lack of interactions. Interactions are defined as deviations from the results expected on the basis of additivity, either dose additivity or response additivity ("no-interactions"-based hypotheses). Ultimately, the various types of interaction and noninteraction can be sorted into three categories: greater-than-additive (synergism, potentiation), additive (additivity, no apparent influence), and less-than-additive (antagonism, inhibition, masking).

The early toxicology literature contains many claims of synergism or antagonism based on study designs that were inadequate to support the claims (Boobis et al. 2011; Borgert et al. 2001; Krishnan and Brodeur 1991). A typical inadequate design might involve exposure to component A and component B at subthreshold exposure levels, and when some biological response to the mixture was observed, a claim of synergism might have been made. However, depending on the individual dose-response relationships for the components, the observed response could be consistent with dose addition (a "no-interactions" hypothesis), greater-than-additive, or less-than-additive joint toxic action (see Appendix A.3.3 for more discussion of evaluating interaction studies). Borgert et al. (2001) presented five criteria that are useful for evaluating toxicological interaction studies and designing valid toxicological tests of interactions:

1. Dose-response curves for the mixture components should be adequately characterized.
2. An appropriate "no-interaction" hypothesis should be explicitly stated and used as the basis for assessing synergy and antagonism.
3. Combination of mixture components should be assessed across a sufficient range (of exposure levels and mixing ratios) to support the goal of the study.
4. Formal statistical tests should be used to distinguish whether the response produced by a dose combination is different (larger or smaller) from that predicted by the 'no-interactions" hypothesis (dose addition or response addition).
5. Interactions should be assessed at relevant levels of biological organizations.

The major mechanisms for toxicant interactions are direct chemical-chemical, pharmacokinetic, and pharmacodynamic mechanisms (Mumtaz and Hertzberg, 1993). Most of these mechanisms affect the internal concentrations of the toxicants or their active forms. Knowledge of these mechanisms for two-chemical (binary) mixtures and for classes of chemicals can be incorporated qualitatively or quantitatively into assessments of mixtures of chemicals using methods described in Chapter 3 of this document.

2. ATSDR APPROACH

ATSDR recommends a 3-tiered approach to evaluating exposure and health effects data to assess public health impacts in communities in the vicinity of sites of chemical or physical agent contamination, such as hazardous waste sites, presenting possible exposures to multiple chemicals and other stressors (Figure 1 presents an overview of the approach). Initial problem formulation activities are followed by as many as three tiers of assessment depending on the availability of exposure and health effects data, the nature of existing exposure and health effects data, and the extent and nature of community concerns for exposure and potential health effects. Parallel assessments are conducted for noncancer effects and cancer (see Figure 1).

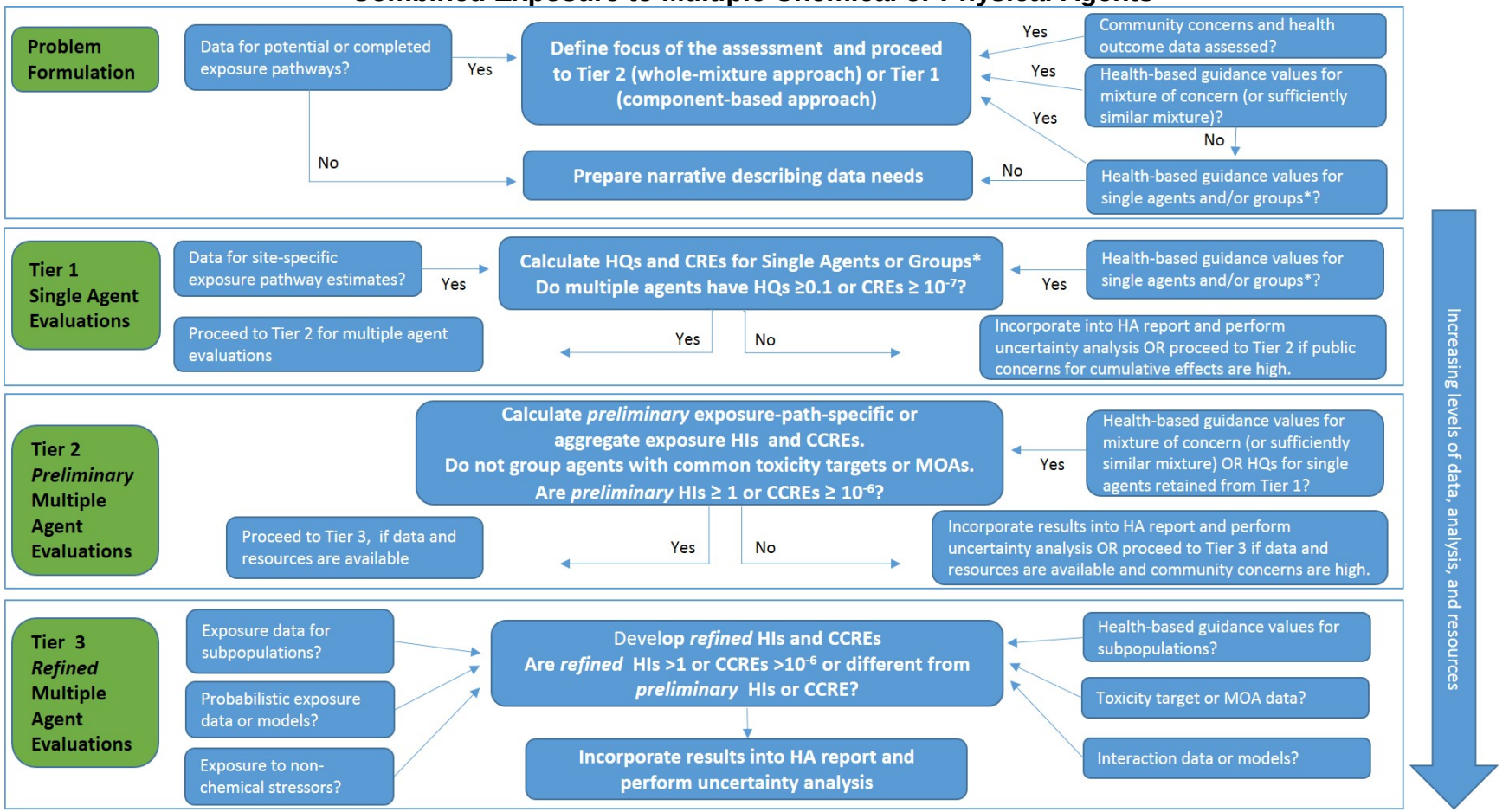
2.1. PROBLEM FORMULATION: SCOPING, PLANNING, DATA COLLECTION

Initial scoping, planning, and data collection activities are essential preliminary steps in preparing a defensible assessment of health impacts associated with contamination of a site with toxic agents (ATSDR 2005a). The initial activities are guided by legislative mandates stating ATSDR consider the following factors in its assessments.

1. *The nature and extent of contamination:* What are the contaminants of concern? What are the temporal and spatial extents of contamination? What media are contaminated (air, water, soil, sediment, food)?
2. *The demographics of exposure:* Who is expected to be exposed (population size)? What potentially susceptible subpopulations may be exposed (e.g., children, elderly, pregnant women)?
3. *The pathways of human exposure (past, present, and future):* How might people be exposed to contaminants (drinking water, eating food, breathing air, having skin contact)? What are site-specific exposure levels for specific populations based on route, frequencies, and duration of exposure and magnitude of media contamination?
4. *Possible health effects associated with site-specific exposure levels:* What toxicologic, epidemiologic, medical, or health outcome data are available to identify possible adverse effects from exposure to contaminants at a site?

The early phases of information gathering and assessment are instrumental in formulation of the problems to be addressed by the health assessment (Figure 1). Initial activities related to the exposure

Figure 1. ATSDR Tiered Approach to Evaluate Exposure and Health Effects Data to Assess Health Impacts from Combined Exposure to Multiple Chemical or Physical Agents



*"Single Agents" can include groups of chemicals with TEFs or RPFs or mixtures with health-based guidance values (e.g., MRLs or cancer slope factors). Groups of chemicals with TEFs or RPFs include dioxins, PAHs, and N-methyl carbamates. Mixtures with ATSDR health-based guidance values include jet fuels (JP-5, JP-8) and PCB mixtures (Aroclor 1254).

ATSDR = Agency for Toxic Substances and Disease Registry; CRE = cancer risk estimate; CCRE = combined cancer risk estimate; HA = health assessment; HI = hazard index; HQ = hazard quotient; MOA = mode of action; MRL = Minimal Risk Level; PAH = polycyclic aromatic hydrocarbon; PCB = polychlorinated biphenyl; RPFs = relative potency factors; TEFs = toxic equivalency factors

evaluation portion of the assessment include collecting site history data, available environmental sampling data, available fate and transport data for the contaminants detected or expected in environmental media, and available exposure and demographics data (ATSDR 2005a). Relevant routes of exposure can be identified at this stage. For example, if residential exposures to contaminated drinking water are of concern, considerations should include exposure by ingestion, inhalation when showering, and dermal exposure when showering. Activities for health effects evaluation include collecting existing health-based guidance values for acceptable levels of contaminants of concern based on toxicologic, epidemiologic, or medical data and any available health outcome data for the community of interest. ATSDR (2005a) also strongly recommends that community health concerns and health outcome data be collected, evaluated, and included in early phases of any health assessment (see Figure 1 Problem Formulation box asking, "Community concerns and health outcome data assessed?").

At this early phase, it is important to establish if the assessment will proceed using a whole-mixture or component-based approach. Ideally, the noncancer and cancer assessments would be based on existing health-based guidance values derived specifically for the site-specific mixture of concern. Only a few complex mixtures have health-based guidance values including certain jet fuels, coke oven emissions, diesel engine exhaust, and PCB mixtures (see Section 3.1). However, it is unlikely that the site-specific mixture of concern will be identical in composition to the tested mixture that is the basis for the guidance value. Alternatively, a whole-mixture approach could be used if: (1) there are high-quality epidemiological data or animal toxicological data available for the site-specific mixture of concern that could be used to derive a health-based exposure value, or (2) the mixture of concern is considered "sufficiently similar" to a tested mixture that is the basis for a guidance value. However, it is also unlikely that appropriate toxicity studies for a site-specific mixture will be available and widely-accepted methods for determining sufficient similarity have not been established (see Section 3.2 for more details regarding establishment of sufficient similarity). Therefore, site-specific health assessments often proceed using component-based approaches (see details in Section 3.3). When using a component-based approach, lumping of certain chemicals or well-defined mixtures within the complex mixture of concern may facilitate the preliminary assessment. For example, well-defined mixtures with health-based guidance values (e.g., JP-8, PCBs) and contaminants that are members of chemical groups with special approved methods for assessing health effects (e.g., relative potency factor [RPF] approach for carcinogenic polycyclic aromatic hydrocarbons [PAHs], toxic equivalency factor [TEF] approach for dioxins, and indicator chemical approach for size classes petroleum hydrocarbons) may be treated as single agents during hazard quotient (HQ) and *preliminary* hazard index calculations (see Tier 1 and 2 evaluations in Figure 1). See Sections 2.2, 2.3, and 3.3 for more details on these approaches.

It is also important to identify any non-chemical agents (e.g., biological, physical, and psychosocial stressors) that may be pertinent to human health during the problem formulation step. At the current time, however, the available scientific information is inadequate to develop specific guidelines for incorporating non-chemical agents in assessment of health impacts from multiple stressors with the exception of cancer risks associated with ionizing radiation (see Section 2.3.2). Therefore, the potential impact of the majority of non-chemical stressors will only be qualitatively examined in health assessments that progress to Tier 3 evaluation (see Section 2.4 for more information). However, it should be noted that combined exposure to noise and ototoxic substances is a recognized emerging risk (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4693596/ and https://www.cdc.gov/niosh/nioshtic-2/20042414.html)

2.2. TIER 1: PRELIMINARY EVALUATION OF EXPOSURE AND HEALTH EFFECTS DATA FOR SINGLE AGENTS AND CHEMICAL MIXTURES WITH HEALTH-BASED GUIDANCE VALUES

2.2.1. Preliminary Exposure Evaluation

To identify chemicals of concern, site history information and environmental sampling data are evaluated to determine whether or not exposure point concentrations can be determined reliably. If appropriate and reliable environmental sampling data are not available, recommendations may be made for filling this critical data gap before proceeding further (ATSDR 2005a). Often, environmental monitoring data are collected by collaborating agencies or companies, such as the EPA or principal responsible parties (PRPs) rarely by ATSDR. The availability of reliable and high quality environmental sampling data, regardless of their source, is essential to the ATSDR public health assessment process.

Information about exposure pathways for contaminants of concern is evaluated next for identifying the following five elements:

1. Source of contamination;
2. Release mechanism into water, soil, air, food, or transfer between media (i.e., fate and transport of contaminants in the environment);
3. Exposure point or area (e.g., drinking water well, soil in a residential yard, sediment in a lake or river);
4. Exposure routes (e.g., ingestion, dermal contact, inhalation);
5. Potentially exposed population (e.g., adult or children residents, clean-up workers).

If all elements are identified, a "completed" exposure pathway exists. If one or more of the elements is missing or uncertain, a "potential" exposure pathway exists. If multiple routes of exposure represent "completed" or "potential" exposure pathways, consideration can be given to aggregate exposures across routes. For completed and potential exposure pathways with appropriate exposure-point concentration data, ATSDR (2005a) conducts preliminary crude and refined screening-level health effects evaluations for each of the individual contaminants of concern (see next section). If no completed or potential exposure pathways are identified, ATSDR (2005a) usually considers that no public health hazards will exist.

2.2.2. Preliminary Health Effects Evaluation

The preliminary health effects evaluation for component-based approaches begins with a crude screening effort in which maximum values of measured (or modeled) environmental media concentrations of individual agents (in air, water, and soil or sediment) are compared to media-specific guideline concentrations for individual agents expected to be safe for the general public (i.e., environmental media comparison values). Comparison values for the crude health effects evaluation include: (1) ATSDR Environmental Media Evaluation Guides (EMEGs), calculated using ATSDR MRLs for noncancer effects from individual agents or defined mixtures of chemicals and standard exposure assumptions; (2) Reference Dose Media Evaluation Guides (RMEGs), calculated using EPA RfDs or RfCs for noncancer effects (when ATSDR chronic noncancer guidance values are not available) and standard exposure assumptions; and (3) Cancer Risk Evaluation Guides (CREGs) calculated using EPA Cancer Oral Slope Factors (OSFs) or Inhalation Unit Risks (IURs) (ATSDR 2005a). Independent and parallel evaluations for noncancer and cancer effects are conducted as illustrated in Figure 1. When appropriate ATSDR or EPA comparison values are not available, ATSDR environmental scientists may select from other health-based guidance values that may be available from other agencies (see ATSDR 2005a for more details). For noncancer effects, individual agents are retained for more refined evaluations of health impacts from individual agents, when site-specific environmental concentrations exceed the EMEGs or RMEGs. ATSDR does not derive cancer slope factors and relies on EPA for their determination. Cancer slope factors are expressed in terms of risk per exposure unit (e.g., risk per mg/kg-day for OSFs or risk per mg/m^3 for IURs), so the product of an exposure estimate and a cancer slope factor yields a cancer risk estimate (CRE). For the crude screening of individual carcinogenic agents, ATSDR multiplies the site-specific environmental concentration by the appropriate EPA cancer slope factor (OSF or IUR) and appropriate default exposure factors for adults (e.g., 2.4 L water/day for 80-kg adults) to arrive at theoretical crude oral or inhalation CREs. When a resultant crude risk estimate exceeds 10^{-6} (one in one million), the agent is retained for more refined evaluation involving site-specific exposure information and consideration of specific subpopulations, such as children or the elderly. When no environmental comparison value is available, a contaminant is retained for refined Tier 1 evaluation with site-specific exposure information, if it has a health-based guidance value, such as an MRL or a cancer OSF or IUR, the assessment proceeds according to the flow chart (Figure 1) (ATSDR 2005a).

The preliminary screening-level health evaluation of single agents ends with a refined evaluation that incorporates site-specific exposure information into the exposure assessment to arrive at administered doses (in units of mg/kg/day for ingestion of water or soil) or concentrations (mg/m^3 in air), which are

compared with health-based guidance values, such as ATSDR MRLs or EPA RfDs/RfCs directly (not EMEGs or RMEGs) in the assessment for noncancer effects or used to calculate CREs from EPA OSFs or IURs (ATSDR 2005a). For noncancer effects, the HQ is the comparative method used. The HQ is the ratio of the exposure estimate to the health-based guidance value. If the exposure estimate is 1 mg Chemical X/kg/day and the MRL is 0.1 mg Chemical X/kg/day, the HQ = (1 mg/kg/day) / (0.1 mg/kg/day) = 10. In Figure 1, the central box for Tier 1 analysis calls for calculation of HQs and CREs for single agents. As described in the Problem Formulation section, the term, "single agents" in Figure 1 is meant to include groups of chemicals with special approved methods for assessing health or mixtures with health-based guidance values (e.g., MRLs). Recommended refined modifications of the hazard index approach can be used to derive HQs for certain groups of chemicals, such as the TEF approach for dioxins and dioxin-like compounds and RPF approaches developed by the EPA Office of Pesticide Programs (OPP) for certain classes of pesticides (organophosphate, carbamate, and pyrethroid insecticides and triazine and chloroacetanillide herbicides). Sections 3.3.5 and Appendix C (Section C.8) of this manual discuss these approaches in more detail.

For sites contaminated with gasoline and other complex petroleum products enriched in hydrocarbons, specific component-based approaches could be used to assess noncancer health impacts (e.g., ASTM 2015; MassDEP 2002; Ohio EPA 2010; Oklahoma DEQ 2012; Total Petroleum Hydrocarbon Criteria Working Group 1997, 1998a, 1998b; Weisman 1998). These approaches involve: (1) lumping the complex mixture into groups of hydrocarbons with similar chemical structures (e.g., aromatic hydrocarbons with 5–9 carbons, aliphatic hydrocarbons with 5–8 carbons); (2) collecting data for concentrations of these groups of hydrocarbons in environmental media; (3) selecting a representative chemical with adequate dose-response data to indicate hazard potential and dose-response relationship for each group (i.e., an indicator chemical); and (4) using the guidance value of the indicator chemical coupled with exposure estimates for all members of the group in the subject mixture to estimate health risk from the group (i.e., calculate class-specific HQs for site-specific exposure pathways). The approach is based on an assumption that toxicity of all detected members of the class can be estimated by the indicator chemical. ATSDR did not derive MRLs for automotive gasoline because of the wide compositional range of formulations for gasoline and the likelihood that components have widely differing environmental fate and transport properties (ATSDR 1995a, 1999; Pohl et al. 1997). Consequently, exposed populations are likely to be exposed to fractions that are not sufficiently similar to the original mixture. The total petroleum hydrocarbon (TPH) approach represents a way to incorporate site-specific environmental data in assessments of potential health impacts for fractions of hydrocarbons in complex petroleum-based products.

Site-specific exposure information to include in the Tier 1 refined exposure assessment can include variability in values for media-specific concentrations, exposure frequencies (days/year), exposure durations (years), intake rates (e.g., liters of water consumed/day or volume of air inhaled/day), or body weights that are different from the crude exposure assumptions. For example, in a crude assessment, the maximum concentration in media samples may have been used, but a refined assessment would consider the variability of concentrations in environmental samples, the frequency of "detects" and non-detects" among the samples, the degree to which concentration variability may be due to spatial variability or "hot spots", and spatial differences in accessibility to the public (ATSDR 2005a).

Chemicals (or defined groups/mixtures treated as single agents) are retained for further in-depth analysis, when the site-specific exposure estimate exceeds the MRL or RfD/RfC (the HQ is $\geq$1) or the CRE is $\geq 10^{-6}$ (see Section 2.2.1). While agents with HQs <1 or CREs $<10^{-6}$ (e.g., 10^{-7} or 10^{-8}) are expected to individually pose no health impacts, they may have an impact when combined exposure to multiple agents is considered. Therefore, all agents with HQs $\geq$0.1 or CREs $\geq 10^{-6}$, are retained for further Tier 2 analysis of noncancer or cancer health impacts from combined exposure to multiple agents (see Figure 1 box asking, "Do multiple agents have HQs $\geq$0.1 or CREs $>10^{-6}$?"). If aggregate exposure is identified as a concern during problem formulation, HQs should be summed across routes of exposure.

2.3. TIER 2: PRELIMINARY ANALYSIS OF EXPOSURE AND HEALTH EFFECTS DATA FOR MULTIPLE AGENTS

Exposure pathways and agents retained in the Tier 1 evaluation can be subjected to further analysis outlined in this (Tier 2) and the following (Tier 3) sections (see Figure 1). The health assessment outcome of these activities is a qualitative narrative description of whether site exposure conditions are of sufficient nature, frequency, and magnitude to adversely impact public health (ATSDR 2005a). The narrative should clearly state what is known and unknown about any of the agents of concern from a site-specific exposure pathway, indicate how the potential for toxic effects from combined exposures to multiple agents at the site was evaluated, and concisely describe the uncertainties in the assessment.

2.3.1. Noncancer Health Impacts from Multiple Agents

Consideration of the potential for toxic effects from combined exposure to multiple agents at a contaminated site is especially important when: 1) Tier 1 evaluations of single agents and chemicals with health guidance values identify multiple agents that approach or exceed MRLs (i.e., the HQ approaches or

exceeds 1); and 2) scoping activities indicate the potential contamination of environmental media (water, soil, or air) with multiple toxic agents from specific processes or products. A hazard index approach is recommended to *preliminarily* evaluate the potential for noncancer toxic effects from combined exposure to multiple agents at a site. As discussed in Section 2.1, the problem formulation phase of a health assessment should determine whether a whole mixture or component-based method should be applied.

For whole mixture approaches, the *preliminary* hazard index would be based on the health-based guidance value for the mixture of concern or a sufficiently similar mixture, rather than on the sum of the HQs for the individual components. It is important to note that the "sufficiently similar" approach would only be a part of the *preliminary* hazard index, if a specific approach has been developed and received widespread review and acceptance. Currently, only qualitative approaches to sufficient similarity determinations have been applied, but statistical approaches are being evaluated and developed (see Section 3.2 for more discussion). One example that could be applied is the ATSDR (2000b) intermediate-duration oral MRL for PCB mixtures that was based on the assumptions that: (1) PCB mixtures are sufficiently similar for dose-response assessment purposes and (2) an MRL based on the lowest LOAELs from studies of specific PCB mixtures (i.e., a simulated environmental mixture and a commercial PCB mixture, Aroclor 1254) would be protective for PCB mixtures in general (see Section 3.2 for more details).

For component-based approaches, the hazard index is based on the assumption of dose addition, and a *preliminary* hazard index is a sum of HQs $\geq$0.1 of all known and measured chemicals for site-specific exposure pathways. The *preliminary* hazard index does not group chemicals based on shared toxicity targets (i.e., common adverse outcomes) or modes of action (MOAs); Tier 3 calls for this type of refinement if data are available and concerns are high for health effects from combined exposure to multiple agents (Figure 1). Chapter 3 of this manual provides more in-depth discussion of the hazard index, the underlying rationale for using the approach, and examples of its applications and modifications.

When a *preliminary* hazard index value exceeds 1, further evaluation is recommended, following guidance for Tier 3 analyses (Figure 1). If aggregate exposure is identified as a concern during problem formulation, *preliminary* hazard indices can be summed across routes of exposure.

2.3.2. Cancer Impacts from Multiple Agents

Public health impacts from combined exposure to multiple carcinogenic agents are assessed with an approach that is separate from, and parallel to, the approach for noncarcinogenic agents (see Section 3.3.6). As discussed in Section 2.1, the problem formulation phase of a health assessment should determine whether a whole mixture or component-based method should be applied.

For whole mixture-based approaches, the *preliminary* combined cancer risk estimate (CCRE) would be based on the cancer slope factor for the mixture of concern or a sufficiently similar mixture, rather than on the sum of the CREs for the individual components. Limitations of the sufficiently similar approach described for noncancer impacts in Section 2.3.1 are applicable to cancer impacts as well.

The component-based approach for cancer, initially recommended by EPA (1986), assumes independent action of carcinogenic agents and adopts the most conservative form of response addition (completely negative correlation of tolerances; i.e., individuals most sensitive to chemical A are least sensitive to chemical B and vice versa; see Appendix A). Individual CREs are calculated by multiplying the site-specific exposure estimate by the EPA cancer slope factor (oral exposure) or IUR for the agent of concern. It is recommended that the combined cancer risk estimates (CCREs) be calculated for all carcinogenic agents identified in site-specific exposure pathways as presenting individual CREs $\geq 10^{-6}$ (see Section 3.3.6). Because EPA Integrated Risk Information System (IRIS) values for slope factors or unit risks are typically upper 95% confidence limit estimates on the lifetime excess cancer risk of individual agents, concern has been raised that summing upper bound risks may lead to unreasonably high estimates of the mixture risk. However, an analysis by Kodell and Chen (1994) suggested that the error in the simple sum of the upper bound risks is small relative to other uncertainties. Furthermore, Cogliano (1997) concluded that the sum of the upper bound risks provides useful information regarding the overall risk from mixtures of carcinogens.

There are embedded variables and assumptions involved in deriving cancer risk estimates for individual carcinogenic agents, as well as the basis for assigning agents to weight-of-evidence (WOE) cancer classification groups. Effective description of these variables and assumptions is important when communicating cancer hazard potential to a community faced with contamination from chemical carcinogens. In the final health assessment, individual and combined cancer risk estimates should be discussed to: (1) qualitatively describe the cancer-causing potentials of identified individual carcinogens and (2) compare site-specific exposure estimates with exposure levels resulting in increased risk for

cancer in toxicology studies or epidemiology studies forming the basis of the slope factors for oral exposure or unit risk estimates for inhalation exposures (ATSDR 2005a). Combined cancer risk estimates from multiple agents in a site-specific exposure pathway in the range of 10^{-6}–10^{-4} or greater are taken to present evidence of a potential health hazard from cancer, but the health assessment narratives should do more than just present estimated risk numbers for individual agents or multiple agents (ATSDR 2005a). CCREs $\geq 10^{-6}$ warrant additional Tier 3 evaluation by including considerations of: (1) the potential for interactions among the identified carcinogenic agents; (2) the relative contributions of specific types of cancer to the combined risk; (3) the relative contributions of individual agents to the overall combined risk; and (4) and the relationship of the CCRE to any community health outcome data and/or concerns.

For the assessment of cancer from ionizing radiation or radionuclides, experts can be consulted to assist in the application of exposure models that estimate radiation dose to specific organs and tissues (ATSDR 2005a). These models and health guidance values for different types of ionizing radiation and radionuclides can be used to calculate CREs for radiation in an analogous approach to the approach for chemical carcinogens. It is recommended that site-specific cancer risk estimates from ionizing radiation and radionuclides be added to those from non-radioactive chemical carcinogens to initially screen for CCREs in site-specific exposure pathways. Estimates of combined cancer risk estimates $\geq 10^{-6}$ warrant additional Tier 3 evaluation. Specific approaches for estimating cancer risks from two classes of chemicals, dioxins and dioxin-like compounds and PAHs, are discussed in more detail in Section 3.3.5. If aggregate exposure is identified as a concern during problem formulation, *preliminary* CCREs should be summed across routes of exposure.

2.4. TIER 3: REFINED ANALYSIS OF EXPOSURE AND HEALTH EFFECTS DATA FOR MULTIPLE AGENTS

Further analysis of exposure and health effects data and other types of data for multiple agents should be conducted when: (1) results of Tier 2 analyses indicate that site-specific exposure pathways have *preliminary* hazard indices ≥ 1 or *preliminary* CCREs $\geq 10^{-6}$; (2) community concerns are high for health effects from multiple site-specific agents of concern; and/or (3) additional community health outcome data provide evidence of health effects from multiple agents (see Figure 1).

Because the dose-additive hazard index approach for noncancer effects and the response-additive approach for adding cancer risks do not account for possible interactions among agents of concern, it is important to determine what is known and unknown about possible greater-than additive or less-than-additive interactions among agents of concern. A first step is to access the information presented in the

Interactions with Other Chemicals sections of the ATSDR Toxicological Profiles for the agents of concern, if available. Section 3.3.1.2 of this framework (*Evidence to Support or Refute the Use of Default Dose-Additivity Approaches*) can also be reviewed. The *in vivo* and *in vitro* research studies reviewed in Section 3.3.1.2 provide support that: (1) dose additivity often provided adequate descriptions of responses to defined mixtures of various classes of chemicals; (2) positive and negative deviations from dose additivity were small from a risk assessment perspective (generally <5-fold); and (3) observed responses to mixtures of chemicals were often below values predicted by dose addition, but higher than values predicted by response addition. Further research may help to confirm or refute the validity of this assumption, in particular for chronic exposure scenarios and for early life exposures with possible later life health outcomes. It is important to understand and communicate that recommended approaches using default assumptions of dose additivity or response additivity are practical tools, which could overestimate or underestimate actual health impacts.

If sufficient data on interaction between mixture components are available, a qualitative WOE approach can be used to evaluate scientific evidence that binary combinations of agents of concern may act in an additive, greater-than-additive or less-than-additive manner (see Section 3.3.4 and Appendix B for more details about this binary weight of evidence [BINWOE] approach). Based on such analysis, qualitative statements can be prepared regarding evidence of interactions that may cause the site-specific exposure pathway hazard index or combined cancer risk estimate to overestimate or underestimate health impacts. The BINWOE approach was used in ATSDR Interaction Profiles for a number of priority chemical mixtures (Pohl and Abadin 2008; Pohl et al. 2003, 2004, 2009; see www.atdr.cdc.gov/interaction profiles). The Interaction Profiles can provide useful recommendations, if the subject mixture components overlap with agents of concern for site-specific exposure pathways.

It is also useful to determine if mixture/interaction physiologically based pharmacokinetic/ pharmacodynamic (PBPK/PD) models exist for combinations of site-specific agents of concern. These types of models have been used to determine external exposure levels at which interactions and deviations from dose additivity may exist (see Section 3.3.7 for more discussion of developed mixture/interaction PBPK and PBPK/PD models and their application).

Because the *preliminary* screening approach for assessing health impacts from exposure to multiple agents is based on simplifying assumptions (e.g., adding HQs or CREs for all agents of concern, regardless of toxicity target or MOA), it can be useful to compare the outcome of the *preliminary* hazard index approach with more refined and stringent applications of the hazard index approach. The most

stringent application requires that all components produce a common effect via a common MOA (this approach is applied by the EPA OPP to produce cumulative risk assessments for various classes of pesticides; see Appendix C, Section C.8), whereas a less stringent application requires that all components produce toxic effects in a common target tissue or organ (see Section 3.3.3 for more details of the Target-organ Toxicity Dose [TTD] modification to the hazard index approach). Comparing the relative magnitudes of exposure-pathway-specific hazard indices calculated for all agents of concern, all agents affecting common toxicity targets, and all agents producing common adverse outcomes via a common MOA can convey a qualitative indicator of the magnitude of uncertainty and the effect of simplifying assumptions on the estimates of potential health impact of multiple agents associated with site-specific exposure pathways. A separate analogous comparison of the *preliminary* CCRE for multiple carcinogenic agents of concern with refined combined estimates based on agents producing common types of cancer and agents producing common types of cancer via common MOAs can also be conducted.

The Tier 3 analysis can also consider probabilistic refinements of exposure models, if appropriate site-specific information is available, and the development of exposure estimates and hazard indices for specific subpopulations of the community that may be more susceptible to the site-specific agents of concern, especially children. Exposure estimates for children and other potentially susceptible populations can be developed as a part of the Tier 1 analysis, using general information on the exposures and potential susceptibility of children and other susceptible populations found in ATSDR toxicological profiles on the agents of concern.

If exposures to physical agents are experienced by communities with contaminated sites and if health guidance values are developed, Tier 3 analyses can: (1) include exposure estimates in site-specific exposure pathways, and (2) calculate individual HQs or individual cancer risks for the physical agent, and 3) add them to the *preliminary* hazard indices or CCREs for site-specific exposure pathways. Currently, the only class of physical agents pertaining to this recommendation is ionizing radiation.

As discussed in Section 3.3.8 of this framework manual, there have been calls for developing guidelines for including nonchemical stressors including biological, physical, and psychosocial stressors in cumulative assessments of health impacts or risks of multiple agents. At the current time, however, the available scientific information is inadequate to develop more specific guidelines for incorporating biological, physical, or psychosocial stressors in assessing health impacts from multiple stressors.

2.5. PRESENTING FINDINGS IN THE HEALTH ASSESSMENT DOCUMENT

Upon completion of the ATSDR 3-tiered approach, assessors should consult Section 8.7 of the ATSDR (2005a) *Public Health Assessment Guidance Manual* for guidance on preparing clear and concise narratives that communicate to the public the findings of the analysis of potential health impacts from single and multiple agents of concern. The health assessment document should contain a narrative of the uncertainties associated with the hazard assessment, regardless of what tier the assessment reached (e.g., uncertainties in exposure modeling, unidentified fractions of the mixture, components with no health effects information, multiple chemicals with HQs near 0.1).

3. OPTIONS AND ISSUES FOR ASSESSING HEALTH IMPACTS OF EXPOSURES TO MULTIPLE CHEMICALS AND OTHER STRESSORS

In general, assessments of health impacts or risks associated with exposures to multiple chemicals and other stressors use one of three approaches: (1) use exposure data and epidemiologic and toxicologic data for the actual mixture of concern; (2) use data for a sufficiently similar mixture; or (3) use data on the components of the mixture. Background information on these approaches is discussed in this chapter in order of preference.

3.1. MIXTURE OF CONCERN APPROACH

When exposure data and health effects data are available for the mixture of concern, use of these data has traditionally been the preferred approach (EPA 1986, 2000, 2003; see Chapter 2 for ATSDR approach and Chapter 4 for recommendations from other agencies). However, data on the mixture of concern are rarely available. When available, such data tend to be for complex mixtures that are considered a health hazard because they are generated in large quantities and are thought to cause adverse health effects. In addition, the exposures of concern generally occur at the source of the mixture.

Examples of complex mixtures with sufficient data for hazard identification and, in some cases, dose-response assessment include coke oven emissions, diesel engine exhaust, and manufactured gas emissions and residues.

- Coke oven emissions were determined by EPA to be carcinogenic to humans based on increased risk of mortality from cancer of the lung, trachea, bronchus, and other tissue sites in workers exposed to coke oven emissions as well as increased tumors in animals exposed by inhalation to aerosols of condensates of coke oven emissions (EPA 1984). Based on an analysis of respiratory cancer mortality data and exposure data for a cohort of steel workers, EPA estimated that lifetime exposure to a concentration of 0.2 $\mu g/m^3$ benzene-soluble organic material from coke oven emissions would produce a 1/100,000 extra risk of dying from respiratory cancer (EPA 1984). Based on recent epidemiological and mechanistic studies, the International Agency for Research on Cancer (IARC) determined in 2012 that sufficient evidence was available to determine that coke production is carcinogenic to humans. IARC's (2012a) determination was based on sufficient evidence for a causal relationship with lung cancer in occupationally exposed workers. IARC (2012a) also determined that there was: (1) sufficient evidence in experimental animals for the carcinogenicity of samples of tar taken from coke ovens; (2) strong evidence for a genotoxic

mechanism involving mutagenic PAHs based on both human and experimental animal studies; and (3) suggestive evidence that multiple mechanisms, including epigenetic mechanisms, may be involved in the carcinogenic response to coke oven emissions.

- Diesel engine exhaust was determined by EPA (2002c) to be likely carcinogenic to humans by inhalation based on: (1) strong, but less than sufficient, evidence for a causal association between diesel exhaust exposure and increased lung cancer risk in workers from varied occupations with diesel exhaust exposure; (2) supporting positive results in genotoxicity tests with diesel exhaust and organic constituents; (3) knowledge that a number of components of diesel exhaust have produced positive results in genotoxicity and carcinogenicity tests; and (4) positive results in cancer bioassays with rodents exposed to high intratracheal instillation doses of whole diesel exhaust, in skin painting studies using extracts of organic whole diesel exhaust, and in many chronic inhalation rat studies showing a positive lung cancer response at high exposures. A quantitative estimate of cancer risk, however, was not developed, because of inadequate exposure-response data from human studies and a determination that doses at which toxicity was observed in rats were much higher than expected environmental exposure levels" (EPA 2002c). A chronic inhalation RfC of 5 $\mu g/m^3$ was derived based on a rat NOAEL of 0.46 mg/m^3 for pulmonary inflammation transformed to a human equivalent concentration using a deposition and clearance model for diesel particulate matter and divided by uncertainty factors of 3 to account for residual interspecies extrapolation uncertainties and 10 for human variability (EPA 2002c). After review of recent epidemiology data, IARC (2014) determined that diesel engine exhaust is carcinogenic to humans based on sufficient evidence in humans indicating a causal relationship with lung cancer and a positive association with urinary bladder cancer. IARC (2014) also determined that there was sufficient evidence for carcinogenicity of whole diesel engine exhaust and particulate matter in experimental animals, but inadequate evidence for carcinogenicity of gas-phase diesel engine exhaust in laboratory animals.

- Occupational exposure during coal gasification was determined by IARC (2010, 2012b) to be carcinogenic to humans based on consistent evidence for increased risk of lung cancer in studies of cohorts of coal gasification workers. Coal gasification workers are expected to be exposed to a wide range of chemicals including asbestos, silica, amines, numerous metals, aliphatic and aromatic hydrocarbons, sulfur dioxide, and aldehydes (IARC 2012b). In support of this determination, IARC also determined that there was: (1) sufficient evidence for the carcinogenicity of coal tars from gas works and manufactured gas plant residues in experimental

animals after dermal or oral exposure; and (2) strong evidence in experimental animals for a genotoxic carcinogenic mechanism involving mutagenic PAHs in coal gasification samples. The EPA IRIS (IRIS 2015) has not assessed the carcinogenicity of occupational exposure during coal gasification or manufactured gas residues.

The advantage of using toxicological or epidemiological data on the mixture of concern to determine a public health guidance value is that any interactions among the components of the mixture should be represented by the health effects data for the original mixture. Limitations of the use of original mixture data include the uncertainties regarding the extent to which the mixture of concern matches the mixture that is the basis for the health guidance value, due to changes in mixture composition with time and distance from the release, and/or differences in the original mixture released into the environment. Thus, for most exposure scenarios, the mixture of concern will likely not be identical to the mixture that is the basis for the health guidance value, even when it is called by the same name (e.g., toxaphene, commercial mixtures of PCBs).

3.2. SUFFICIENTLY SIMILAR MIXTURE APPROACH

If no adequate data are available on the mixture of concern, but health effects data or guidance values are available on a sufficiently similar mixture, the health hazard assessment may be based on the health effects data for the sufficiently similar mixture (see Chapter 2; EPA 1986, 2000, 2003).

Sufficiently similar mixtures are those having the same chemicals but in different proportions, or having most, but not all, chemicals in common and in similar proportions. In addition, sufficiently similar mixtures and their components have similar fate, transport, and health effects, whereas dissimilar mixtures do not.

ATSDR recommends the qualitative approach used by EPA (2000) in determining (or providing support for an assumption of) sufficient similarity. The approach considers the following criteria:

1. establish that common effects or common effects mediated by a common MOA are caused by short- or long-term exposure to the mixtures or their principal components;
2. identify common components across the mixtures in similar proportions;
3. establish a common source or process of formation across the mixtures; and

4. consider the results of time-dependent transformations of mixtures introduced into the environment.

3.2.1. Examples of Sufficient Similarity Approaches used for Hazard Identification and Dose-response Assessments

Discussion follows of several examples of mixtures that have been variously (not systematically) evaluated for sufficient similarity for hazard identification and dose-response assessment purposes: jet fuels (ATSDR 2015), gasolines (ASTM 2015; ATSDR 1995a, 1999; MassDEP 2002; Ohio EPA 2010; Oklahoma DEQ 2012; Total Petroleum Hydrocarbon Criteria Working Group 1997, 1998a, 1998b; Weisman 1998), PCB mixtures (ATSDR 2000b; EPA 1996; IARC 2015), and comparative potency method for combustion products (Albert et al. 1983; Lewtas 1985, 1988).

Jet fuels (JP-5, JP-8, and Jet A) are kerosene-based fuels refined from crude or shale-derived oil by straight or catalyst-assisted distillation (ATSDR 2015). Jet fuels are generally refined under more stringent performance-related conditions than kerosene and contain >200 aliphatic and aromatic hydrocarbons (C_6–C_{17}) as principal components, as well as additives (such as antioxidants, corrosion inhibitors, and biocides) that can vary from one fuel type to the other (ATSDR 2015). In 1998, ATSDR derived an intermediate-duration inhalation MRL for both JP-5 and JP-8 based on a study identifying liver effects in rats exposed by inhalation to JP-5 vapor (ATSDR 1998a). Although a formal consideration of sufficient similarity was not conducted in 1998, the recommendation to use the JP-5-based MRL for JP-8 exposures was based on a sufficient similarity assumption. ATSDR derived updated MRLs for jet fuels in 2013 using more recent animal toxicology studies indicating that jet fuels may not produce common critical effects (ATSDR 2015). ATSDR derived separate intermediate-duration inhalation MRLs for JP-5 (based on liver effects in rats) and JP-8 (based on neurotoxic effects in rats) and did not derive one for Jet A due to inadequate data. For oral exposures, ATSDR derived acute- and intermediate-duration oral MRLs for JP-8 (based on immunotoxic effects in mice), but no oral MRLs for JP-5 or Jet A due to inadequate data. The 2015 ATSDR Toxicological Profile did not directly discuss the possible utilization of MRLs based on data for one jet fuel type as surrogates (i.e., sufficiently similar mixtures) for jet fuels with inadequate data (ATSDR 2015).

ATSDR did not derive MRLs for automotive gasoline because of the wide compositional range of formulations for gasoline and the likelihood that components have widely differing environmental fate and transport properties (ATSDR 1995a, 1999; Pohl et al. 1997). This decision represented an application of several of the recommended qualitative determination criteria listed above. Consequently, exposed populations are likely to be exposed to fractions that are not sufficiently similar to the original

mixture. Parallel to this determination, other agencies have recommended modified component-based approaches to assessing risks from sites contaminated with gasoline and other petroleum products (e.g., ASTM 2015; MassDEP 2002; Ohio EPA 2010; Oklahoma DEQ 2012; Total Petroleum Hydrocarbon Criteria Working Group 1997, 1998a, 1998b; Weisman 1998). These approaches involve separating the complex mixture into groups of chemicals with similar chemical structures (e.g., aromatic hydrocarbons with 5–9 carbons, aliphatic hydrocarbons with 5–8 carbons), selecting a representative chemical with adequate dose-response data to indicate hazard potential and dose-response relationship for each group (i.e., an indicator chemical), and using the guidance value of the indicator chemical coupled with exposure estimates for all members of the group in the subject mixture to estimate health risk from the group.

PCBs are a class of 209 aromatic congeners, each containing 1–10 chlorines attached to the core biphenyl molecule (ATSDR 2000b; IARC 2015). Commercial PCB mixtures, previously used as coolants in electrical capacitors and transformers, were mixtures of many PCB congeners of variable composition (ATSDR 2000b; IARC 2015). The composition of environmental PCB mixtures can be different from that of commercial mixtures, because rates of weathering and biotransformation vary across PCB congeners and environmental conditions (ATSDR 2000b; IARC 2015). Discussion follows of three examples of assessments made for PCB mixtures that are variably dependent on an assumption of sufficient similarity.

IARC (2015) determined that PCBs in general and dioxin-like PCBs (PCBs that produce toxic effects through aryl hydrocarbon receptor mediation) are carcinogenic to humans, based on sufficient evidence of carcinogenicity from >70 epidemiology studies of PCB mixture-exposed workers and studies of experimental animals exposed to individual PCB congeners, commercial PCB mixtures, or synthetic mixtures of various PCB congeners, including simulated environmental mixtures of PCB congeners. Based on mechanistic data, IARC (2015) determined that individual PCBs cause cancer through multiple mechanisms and that the carcinogenicity of PCB mixtures cannot be solely attributed to the dioxin-like PCBs.

ATSDR derived an intermediate-duration oral MRL for PCB mixtures based on a LOAEL for neurobehavioral changes in infant monkeys exposed to a simulated environmental PCB mixture containing 80% of the congeners typically found in human breast milk samples (i.e., a simulated environmental mixture) and a chronic-duration oral MRL based on a LOAEL for immunological effects in adult monkeys fed encapsulated doses of a commercial PCB mixture (Aroclor 1254) for 23–55 months

(ATSDR 2000b). ATSDR acknowledged the compositional variation among commercial and environmental PCB mixtures, but derived oral MRLs for PCB mixtures by evaluating all available toxicity studies on commercial and synthetic PCB mixtures and selecting the studies with the lowest LOAEL values as the basis of the MRLs. Inherent in this process are the assumptions that PCB mixtures are sufficiently similar for dose-response assessment purposes and basing the MRLs on the lowest LOAELs from studies of specific PCB mixtures (i.e., a simulated environmental mixture and Aroclor 1254) would be protective for PCB mixtures in general. In support of the latter assumption, ATSDR noted that the chronic MRL based on immunological effects in adult monkeys was similar to a chronic MRL based on an estimated NOAEL for the lack of developmental effects in a study of children of North Carolina women exposed to environmental PCB mixtures as indicated by PCB levels in breast milk samples (ATSDR 2000b).

In a cancer dose-response assessment for PCB mixtures, EPA (1996) recommended a tiered risk assessment approach that used different slope factors (based on cancer bioassays with different commercial PCB mixtures differing in chlorine content) for different exposure scenarios. Exposure scenarios were grouped in consideration of how environmental processes influence the distribution of PCB congeners in environmental media. This recommendation was based on several pieces of evidence, including: (1) environmental PCB mixtures are expected to present increased risk for cancer because the compositional range of PCB congeners in commercial mixtures overlaps with the range in environmental PCB mixtures; (2) higher chlorinated congeners tend to be more potent and more persistent in soils and sediments than lower chlorinated congeners; (3) PCB congeners found in water or air tend to be lower in chlorine content than congeners found in soil, sediment, and tissues of animal species high in the food chain; and (4) bioaccumulative, high-chlorine content PCB congeners appear to be more potent carcinogens than commercial Aroclor mixtures. Individual human oral cancer slope factors were derived from tumor incidence data from five 2-year bioassays with rats exposed to one of four Aroclor PCB mixtures (1016, 1242, 1254, and 1260) varying in percentage chlorine content (41, 42, 54, and 60%, respectively). The OSFs (95^{th} upper confidence limits on slope in units of risk per mg/kg/day) were: 0.07 (Aroclor 1016); 0.4 (Aroclor 1242); 1.5 (Aroclor 1254); 0.5 (Aroclor 1260); and 2.2 (Aroclor 1260). A composite OSF of 2 per mg/kg/day was recommended for high risk and persistence exposures including food-chain exposure, sediment or soil ingestion, dust or aerosol inhalation, and early-life exposures by any pathway. A composite OSF of 0.4 per mg/kg/day was recommended for low risk and persistence exposures, including ingestion of water-soluble congeners, inhalation of evaporated congeners, and dermal exposures. The lowest slope factor of 0.07 per mg/kg-day was recommended for exposure to PCB mixtures containing PCB congeners with more than four chlorines, accounting for <0.05% of total PCBs.

The comparative potency method uses data for a set of similar mixtures to estimate a scaling factor that relates cancer potency derived from a chronic animal study or human epidemiology study to potency in a mouse skin painting assay. The cancer potency factor for an additional similar mixture for which only data from the skin painting assay are available can be estimated using this scaling factor (Calabrese 1991; EPA 2000; Hertzberg et al. 1999; NRC 1988). This method was used in the estimation of human cancer risk from very complex mixtures of combustion emissions from various sources (Albert et al. 1983; Lewtas 1985, 1988), but it has not been applied in site-specific public health or risk assessments.

3.2.2. Future Approaches to Sufficient Similarity Assessments

Recent methods have been proposed for determining sufficient similarity among mixtures of pyrethroid insecticides found in surface wipe samples from U.S. child care centers (Marshall et al. 2013) and mixtures of disinfection byproducts in drinking water (Feder et al. 2009a, 2009b). These methods require additional review across expert panels and regulatory and public health agencies before they are widely accepted.

Marshall et al. (2013) developed a statistical method for assessing sufficient similarity of mixtures of up to 15 pyrethroid insecticides detected in floor wipe samples collected in 2001 from multiple locations in 168 U.S. licensed child care centers, relative to a reference mixture (of five pyrethroids) with dose-response data from an acute, oral exposure study of a neurological end point (motor activity) in rats (Wolansky et al. 2009). In this analysis, each floor-wipe sample was considered a unique mixture without any dose-response data. The composition and mixing ratio of the five-pyrethroid reference mixture was based on the average percent composition of the six most prevalent components in the floor-wipe samples with the top 10% highest total pyrethroid concentrations; the most prevalent components included cis- and trans-permethrin, which were combined into one-component (permethrins) in the five-component reference mixture. The method used to test whether or not any of the floor-wipe "mixtures" were sufficiently similar to the reference mixture was a modification of a statistical method described by Stork et al. (2008), which uses equivalence testing methodology comparing Euclidean distances between benchmark dose (BMD) estimates for different mixtures. Because the floor-wipe mixture did not have dose-response data to derive BMDs, a modification of the Stork et al. (2008) method was made, based on an assumption that BMDs for the "floor-wipe mixtures" could be estimated from the proportions of the analyzed 15 pyrethroids in them and the BMD for the reference mixture. Among the 168 floor-wipe samples, 42 had concentrations for each of the 15 pyrethroids that were below the detection limit. For the

remaining 126 floor-wipe samples, seven were determined to be sufficiently similar to the reference mixture using the modified method. In a subsequent analysis that adjusted the floor-wipe-mixture estimated BMDs and the BMD for the reference mixture by relative potency factors for the individual pyrethroids, 114/126 floor-wipe samples were determined to be sufficiently similar to the reference mixture.

Feder et al. (2009a, 2009b) applied multivariate statistical procedures to a data set describing chemical composition variables associated with disinfection processes and mutagenic activities of samples of finished water and distribution system water from five water treatment plants. The analysis included six chemical characteristics (total organic carbon, total organic halogens, total trihalomethanes, six haloacetic acids, percent brominated total trihalomethanes, and percent brominated haloacetic acids) and mutagenic activities of the samples. The statistical analysis indicated that the finished (post treatment) samples from the groundwater treatment plant were significantly different from the finished samples from the four surface water treatment plants, and that differences among the four surface water treatment plants were less clearly indicated. The four samples were sufficiently similar mixtures

Other future applications to predict high, medium, or low cancer potencies of very complex mixtures may involve predictions from analyses of gene expression profiles from short-term exposure (Tilton et al. 2015). Tilton et al. (2015) analyzed gene expression profiles in skin of mice collected 12 hours after applying tumor-initiating doses of individual PAHs (benzo[a]pyrene or dibenzo[def,p] chrysene) or very complex PAH-containing mixtures (diesel particulate extracts, coal tar extracts, or cigarette smoke condensate) and compared the results of the analysis with tumor outcomes. The analyses identified short-term-initiated biological signaling pathways that were predictive of tumor initiation potency classified as high, medium, or low (Tilton et al. 2015).

A more quantitative experimental basis to the determination of sufficient similarity among complex mixtures involves: (1) advanced chemical analytical capabilities (e.g., gas chromatography [GC]/mass spectrometry [MS], GC/flame ionization detection, and 2-dimensional GC); (2) statistical techniques for pattern recognition and principal component analysis; and (3) multivariate regression techniques to link chemical components with activity in biological tests (Eide et al. 2002, 2004; Feder et al. 2009a, 2009b; Teuschler 2007; Tian et al. 2015; Ventura et al. 2011). For example, Eide et al. (2002, 2004) used principal component analysis to analyze GC/MS data for 20–33 samples of organic extracts of exhaust particles collected from diesel engines, gas-fired furnaces, or fuel oil furnaces, followed by multivariate regression analysis to correlate the compositional data with measured activities of the samples in the

Ames mutagenicity test. A regression model was developed that identified compounds in the mixtures that co-varied with biological activity. The regression model could be used to predict mutagenicity of another exhaust particle sample with GC/MS data and provide quantitative information to determine sufficient similarity of two or more exhaust particle samples. Tian et al. (2015) used a similar approach to relate *in vitro* Chinese hamster ovary cell cytotoxicity of 40 samples of complex mixtures of organic chemicals extracted from polluted water in Shenqiu County of the Huai River basin in China with compositional data from GC/MS. A regression model was constructed from training datasets from 32 of the 40 samples. The model was used to compare predicted and observed cytotoxicity values obtained from eight "test" samples. The model explained about 92% of the cytotoxicity variability in the training data set, but only about 40% in the test data sets. This result suggests an inadequacy of the model to explain the variability in the test data sets and indicates compositional or biological activity dissimilarities between the training and test data samples (Tian et al. 2015).

Another research effort has developed a series of statistical screening models to predict several types of toxic end points (general, developmental, reproductive, and genetic toxicity) based on the polycyclic aromatic compound contents of a class of complex petroleum-derived mixtures called high-boiling petroleum substances (HBPS) (Gray et al. 2013; McKee et al. 2013; Murray et al. 2013a, 2013b; Nicolich et al. 2013; Roth et al. 2013) HBPS are complex mixtures typically composed of thousands of chemicals with final boiling points ≥~650°F (Gray et al. 2013). HBPS include various substances from petroleum refining streams called asphalts, aromatic extracts, crude oils, gas oils, heavy fuel oils, lubricating oil basestock, waxes, and residual hydrocarbon wastes (Gray et al. 2013). The composition of HBPS (even those with the same name) can vary due to compositional variations in crude oil starting materials, refining conditions, and product specifications (Gray et al. 2013). In a seminal research report examining systemic and developmental effects following repeated dermal exposure of rats to a number of HBPS (gas oils, heavy fuel oil components, and distillate aromatic extracts), common outcomes included increased liver weight, decreased thymus weight, decreased blood end points, increased resorption frequency, and decreased fetal weight (Feuston et al. 1994). The lowest-observed-effect levels in these studies were correlated (Spearman rank test) with the polycyclic aromatic compound weight percent of dimethyl sulfoxide (DMSO) extracts of the test HBPS samples, providing evidence that the polycyclic aromatic compound components were related to the effects of the original mixtures. Subsequent efforts used data from 39 dermal toxicity studies of HBPS samples with polycyclic aromatic compound compositional data to develop predictive models for repeated dose and developmental toxicity end points (Murray et al. 2013a, 2013b; Nicolich et al. 2013; Roth et al. 2013) and data from bacterial mutagenicity assays from 193 samples from several types of HBPS to develop predictive models for mutagenicity end points

(McKee et al. 2013). The statistical correlation models linearly regressed four repeated-dose end points (absolute thymus weight, hemoglobin count, platelet count, and relative liver weight) and three developmental end points (liver fetus count, fetal weight, and percent resorptions) against polycyclic aromatic compound contents (and other explanatory variables) (Nicolich et al. 2013). Several analytical measures of polycyclic aromatic compound contents were investigated as explanatory variables for the models, but the best fits were obtained with weight-percent of DMSO-extracts in seven aromatic ring classes (1, 2, 3, 4, 5, 6, and 7 and more rings) and other biological variables (such as body weight, gender, and duration of exposure for the systemic repeated dose end points and control group values for the developmental end points) (Murray et al. 2013a; Roth et al. 2013). Correlations ("r values) between observed doses associated with specific responses and values predicted with the final models were >0.9 (Nicolich et al. 2013). The authors suggested that the systemic and developmental toxicity models would be useful for screening untested complex mixture samples with polycyclic aromatic compound compositional data for setting priorities for further biological testing (Nicolich et al. 2013). They further noted that the data should not be used to draw conclusions about whether the polycyclic aromatic compound content is the cause of the repeated-dose or developmental toxicity, only that the polycyclic aromatic compound content of a petroleum substance may allow an estimate of toxicity through modeling. The statistical model developed to predict the general mutagenic outcome for HBPS samples in a modified *Salmonella* assay based on the polycyclic aromatic compound content of the samples predicted the mutagenic outcome of 99% of the 193 data sets used to develop the model and 94% of 49 data sets not used to develop the model (McKee et al. 2013). The general outcome used a concept termed the "mutagenicity index," defined as the slope of the initial portion of the dose-response curve. The model predicted a final binary mutagenicity index outcome as either <1 or ≥ 1.

3.2.3. Current Limitations to Sufficient Similarity Approaches

As discussed earlier in this chapter, determination of sufficient similarity between mixtures often requires qualitative judgement after evaluation of available information on the chemical composition and biological activities of any two or more mixtures. Chemical information alone may not be sufficient to have confidence in a determination of sufficient similarity for hazard identification or dose-response purposes, especially for complex mixtures like engine exhausts, wood preserving wastes, coal tars, or manufactured gas waste residues (Cizmas et al. 2004; DeMarini et al. 1989; Simmons and Berman 1989). Even with data on comparative biological activities, determination and validation of sufficient similarity among complex mixtures is challenging due to: (1) variable source and weathering conditions that can influence chemical composition; (2) unidentified and variable toxic chemicals in the mixtures; and (3)

unexpected or unknown interactions that may occur among mixture components (Cizmas et al. 2004; Eide et al. 2002, 2004; Rice et al. 2009; Teuschler 2007). For example, in a study of two wood preserving waste mixtures containing PAHs, pentachlorophenol, and other chemicals, the order of the observed activities of fractionated crude extracts of the mixtures in *in vitro* genotoxicity tests were not well correlated with the activities that were expected based on chemical composition and relative potencies of the known components (Cizmas et al. 2004).

3.3. COMPONENT-BASED APPROACHES

3.3.1. Issues Related to Component-Based Approaches

3.3.1.1 Concepts of Additivity

Due to the lack of suitable health criteria for the mixture of concern or a sufficiently similar mixture, approaches involving the components of a mixture are commonly used for mixtures associated with contaminated sites. These methods are based on an assumption that the exposures or the responses to the mixture components are additive. The classical statistical concepts of dose addition and response addition are based on assumptions of common or different modes or mechanisms of action, respectively (Bliss 1939; Finney 1971), whereas a more generalized dose-addition concept proposed by Berenbaum (1985) and Gennings et al. (2005) does not require assumptions about mechanisms of action.

Dose Addition. Dose addition, also known as *concentration addition*, *simple similar action*, and *similar joint action*, assumes that the components of a mixture behave as concentrations or dilutions of one another, differing only in their potencies (Bliss 1939; Finney 1971; Loewe and Muischnek 1926). The dose-response curves are parallel (i.e., the regression lines of probits on log doses are parallel), and tolerance (or susceptibility) to the components is completely positively correlated (the organisms most susceptible to chemical A also will be most susceptible to chemical B). The response to the mixture can be predicted by summing the doses of the components after adjusting for the differences in potencies. Dose addition is considered most appropriate for mixtures with components that affect the same end point by the same MOA (EPA 1986, 1988, 2000). It has been suggested that the requirement for parallel dose-response curves and complete correlation of tolerances may be too stringent (e.g., Plackett and Hewlett 1952; Svendsgaard and Hertzberg 1994), and that in the low-dose region in which the response is linear, dose additivity may hold for independently acting chemicals as well (Svendsgaard and Hertzberg 1994). Dose addition is the underlying assumption of the hazard index method, and the TEF and RPF approaches (Sections 3.3.2 and 3.3.5).

Response Addition. Response addition, also known as *simple independent action* and *independent joint action* (Bliss 1939), assumes that the chemicals act independently and by different MOAs. Three mathematical definitions of response addition have been described based on the direction and degree to which the distribution of tolerance (or susceptibility) to one component may or may not be correlated with the distribution of tolerance to another component. When tolerances are completely positively correlated (r=+1), the order of individual tolerances to chemical A are identical to that of individual tolerances to chemical B. When the tolerances are completely negatively correlated (r=-1), the orders of individual tolerances to chemicals A and B are directly opposite. The third condition for the mathematical description of response addition is when there is no correlation (r=0) between the order of individual tolerances to chemicals A and B. The response-additive equations estimate the response to a mixture from the probabilities of response to the individual components and the conditional correlation of tolerances. Response addition is the underlying assumption of: (1) an approach to cancer risk assessment for components of mixtures at Superfund sites (EPA 1989a); (2) EPA's (EPA 2000) and ATSDR's default screening-level approach to noncancer health assessment for components with dissimilar toxicity targets, when whole-mixture data and interaction data are not available and exposure levels for components are below guidance values (RfCs, RfDs, or MRLs); and (3) the American Conference of Governmental Industrial Hygienists (ACGIH) approach to assessing the hazard of occupational exposure to agents that act independently (see Section 3.3.6 and Appendix C, Section C.1).

Generalized Dose Addition. Berenbaum (1985) described a general definition of additivity, which does not require chemicals in a mixture to have a common mechanism of action and which Gennings et al. (2005) algebraically related to statistical additivity models to be used in a method for assessing toxicological interactions in mixtures. In the statistical additivity models, when the rate of change in response of a chemical in a mixture (i.e., the slope of the dose-response relationship) does not change in response to other chemicals in the mixture, the chemical is claimed to act additively with the other chemicals (i.e., no interaction occurs). When the slope changes, an interaction (a deviation from additivity) is claimed. The method described by Gennings et al. (2002, 2004, 2005) requires descriptive dose-response data for each individual chemical in the mixture, as well as dose-response data for the mixture, but does not require the assumption of common mechanism of action or common adverse outcome for mixture components. This method was used to determine whether or not deviations from additivity occurred in responses of serum thyroxine levels in rats given four daily gavage doses of an 18-component mixture of polyhalogenated aromatic compounds at six dose levels (Crofton et al. 2005).

On theoretical grounds, Bosgra et al. (2009) questioned the general applicability of Berenbaum's general definition of additivity and provided an example of a biochemical mechanism in which two chemicals do not interact, but for which methods based on Berenbaum's definition would predict interaction. Bosgra et al. (2009) recognized the pragmatic usefulness of statistical methods based on Berenbaum's general definition in empirically assessing joint toxic action of chemicals in a mixture, but warned that deviations from additivity with these methods are incapable of defining specific biochemical mechanisms of interaction.

Dose Addition as a Public Health Protective Action. An underlying public health protective impetus of recommendations for screening level dose additivity is demonstrated by considering exposure to two theoretical chemicals, A and B, at exposure levels (0.9 units for A and 9 units for B), slightly below their respective toxicity guidance values (such as MRLs or RfDs) of 1 and 10 units (see also Appendix A for further illustrations). Response addition for the condition of completely negative correlations between susceptibilities to A and B predicts that exposure to A and B at these subtoxic levels would not produce an adverse effect [(response to A + B) = (response to A = 0) + (response to B = 0) = 0], but dose addition, using the concept of adding HQs (the exposure level to an agent divided by its toxicity guidance value), would indicate a concern for health hazard (hazard index = HQ A + HQ B = (0.9/1) + (9/10) = 0.9 + 0.9 = 1.8; see next section for further discussion of the hazard index approach, where HQs and hazard indices <1 indicate no hazard, and HQs and hazard indices >1 indicate increased risk for hazard).

Additional detail regarding dose addition and response addition is provided in Appendix A.

3.3.1.2 Evidence to Support or Refute the Use of Default Dose-Additivity Approaches

Until 1991, most published toxicological studies of possible interactions among environmental chemicals involved only pairs of chemicals (Hertzberg and Teuschler 2002; Krishnan and Brodeur 1991). Although the principles for statistically assessing deviations from additivity (either dose addition or response addition) had long been laid out in the published literature (e.g., Bliss 1939; Loewe and Muischnek 1926), many published toxicological studies on binary mixtures of environmental chemicals or drugs claiming to provide evidence for synergy or antagonism were inadequately designed to support the claims (Berenbaum 1989, 1990; Boobis et al. 2011; Borgert et al. 2001; Hertzberg and Teuschler 2002; Krishnan and Brodeur 1991). Most of the studies lacked suitable designs to conduct formal statistical tests to determine whether the responses to the mixture were different from the "no-interaction" hypotheses of dose additivity or response additivity. However, several early studies using overtly toxic acute doses of

binary mixtures showed that deviations from dose additivity were generally less than a factor of 5 (e.g., Smyth et al. 1969, 1970; Withey and Hall 1975; see Appendix A for more discussion). Likewise, toxicity studies on guppies and frogs using mixtures of 3 to as many as 50 components also tended to indicate that deviations from dose addition were not substantial (e.g., Dawson 1994; Hermens et al. 1985; Konemann 1981). Other studies were designed to test whether or not adverse effects could be observed when components of four- to nine-component mixtures were at NOAELs, but these studies lacked suitable designs to conduct formal statistical tests to determine whether the responses to the mixture were different from the "no-interaction" hypotheses of dose additivity or response additivity. Dose-additivity appeared to adequately describe the toxic action of a mixture of four kidney toxicants with a common MOA in rats fed a mixture of the four components in food for 4 weeks (Feron et al. 1995), whereas less-than-dose additivity or response additivity appeared to adequately describe toxic actions of mixtures of eight (Jonker et al. 1990) or nine (Groten et al. 1997) chemicals with dissimilar MOAs and targets, or a mixture of four kidney toxicants with dissimilar MOAs (Jonker et al. 1993). These types of observations have been used to support recommendations to use screening-level, component-based, dose-addition approaches for mixtures of chemicals having common MOAs or common adverse outcomes or target organs (e.g., this framework and ACGIH 2015; EPA 1986, 2000, 2002b, 2003; Meek et al. 2011, NRC 2004b). For mixtures of chemicals not having common MOAs or common adverse outcomes or target organs, some organizations recommend screening-level, component-based approaches based on response addition (i.e., independent action) (ACGIH 2015; EPA 2000).

Results from in vivo Studies. A number of animal studies conducted after the seminal review by Krishnan and Brodeur (1991) have used adequate designs to examine whether or not dose addition or response addition provided adequate descriptions of dose-response data for defined mixtures of more than two environmental chemicals having common MOAs or common adverse outcomes and to determine whether or not there were interactions among the components (Borgert et al. 2012; Cao et al. 2011; Christiansen et al. 2009; Crofton et al. 2005; EPA 2006b, 2007b, 2011b; Fattore et al. 2000; Gao et al. 1999; Gennings et al. 2002; Hamm et al. 2003; Hass et al. 2007; Hertzberg et al. 2013; Howdeshell et al. 2008; Jarvis et al. 2014; Moser et al. 2005, 2006, 2012; Nesnow et al. 1998; NRC 2008; Padilla 2006; Rider et al. 2008; Starr et al. 2012; Tajima et al. 2002;Van den Berg et al. 2006; Walker et al. 2005; Wolansky et al. 2009). In the context of these studies (summaries of results follow) and this framework, interactions among components of a mixture are defined as deviations from what would be expected if there were no interactions. If dose addition is the expected "no interaction" model, then observations greater than responses predicted by dose additivity are synonymous with synergy and observations less than predicted responses are synonymous with antagonism.

- Greater-than-response additive effects (synergy) at lower doses and less-than-response additive effects (antagonism) at higher doses were observed in lung tumor responses in mice exposed to single intraperitoneal injections of two dose levels of a mixture of five nonsubstituted PAHs at ratios similar to ratios in environmental air and combustion samples (Nesnow et al. 1998). The experimental design was a 2^5 factorial, 32-dose group scheme yielding lung adenoma per mouse data (8 months after dose administration). A response surface model based on response addition was used to predict lung tumor responses to compare with observed responses for each of the 32 "quintary" dose groups. Deviations from response additivity were small and less than about 2-fold different from the response additivity predictions. Earlier *in vivo* and *in vitro* studies of binary combinations of PAHs in producing cancer or cancer-related effects provided conflicting evidence for both greater-than-additive and less-than-additive interactions, depending on the evaluated compounds, examined end points, and test system, although most of these studies were not adequately designed to statistically test for consistency with dose additivity or response additivity (Jarvis et al. 2014; Nesnow et al. 1998). Possible contributing factors to deviations from additivity have been proposed, such as competitive inhibition or differential induction of bioactivating or detoxifying enzymes, but definitive conclusions about the underlying mechanisms of biochemical interactions among PAHs cannot be drawn because of the complexity of bioactivation and detoxification mechanisms and the complexity of the development of cancer (Jarvis et al. 2014; Nesnow et al. 1998).

- Greater-than-dose-additive effects were observed on several neurological end points in adult or weanling rats orally exposed to mixtures of five or four organophosphorus insecticides at relative proportions similar to those observed in the U.S. diet (Moser et al. 2005, 2006; Padilla 2006). Comparison of predicted (using a dose-additive model based on dose-response relationships for the individual pesticides) and empirical ED_{20} and ED_{50} values for the end points indicated that the greater-than-additive effects were small, from about 1.2–3-fold in magnitude. Earlier studies of the lethality of 43 pairs of organophosphorus insecticides in rats indicated that dose additivity explained 21 pairs, 18 pairs showed less-than-additive effects, and only 4 pairs showed greater-than-additive effects (Dubois 1961). The EPA (2006b) cumulative risk assessment for organophosphorus insecticides concluded that dose addition is a reasonable approach for estimating cumulative risk of mixtures of organophosphorus insecticides, and that the available data did not provide a sufficient basis to depart from dose additivity, based on: (1) these data; (2) other data indicating that toxicokinetic interactions between organophosphorus insecticides

can be complex; and (3) evidence that organophosphorus insecticides have a common mechanism of action (cholinesterase inhibition) in producing neurological effects.

- Dose additivity adequately explained adverse neurological end points (e.g., brain cholinesterase activity and motor activity) in adult rats given single oral doses at five levels of a seven-component mixture of N-methyl carbamates (EPA 2007b). The mixture was designed to deliver equipotent contributions by the components to brain cholinesterase inhibition based on dose-response relationships characterized for each component alone. Ninety-five percent confidence intervals for brain cholinesterase activities predicted by dose additivity overlapped with observed values. The EPA (2007b) cumulative risk assessment for N-methyl carbamates concluded that dose additivity for cumulative risk assessment of mixtures of these pesticides is "reasonable" for this group of insecticides representing a common mechanism of action group (cholinesterase inhibition by a different mechanism than organophosphorus insecticides). An expanded study examined motor activity and cholinesterase activities (brain and red blood cells) in adult and weanling rats given single oral doses of five levels of the seven-component equipotent mixture or another environmentally relevant mixture containing the same components in a different mixing ratio based on California sales data for these pesticides (Moser et al. 2012). Using a statistical approach described by Hertzberg et al. (2013), the equipotent mixture results for adult rats showed dose additivity for red blood cell cholinesterase and motor activity and greater-than-dose additivity for brain cholinesterase at a middle dose level only; for weanling rats, brain cholinesterase and motor activity were dose additive and red blood cell cholinesterase was slightly less-than-dose additive. Exposure of both ages to the other mixture showed greater than dose additivity (synergy) on all three end points, but the magnitude of deviation from dose additivity was small, ranging from 1.5- to 2.6-fold for the different end points in the two ages of rat (Moser et al. 2012). The results also indicate that interactions (i.e., deviations from dose additivity) can be dependent on end point examined, age or stage of development, and mixing ratios of components.

- Dose additivity adequately explained the joint action of mixtures of 11 pyrethroid insecticides (Wolansky et al. 2009) or 5 pyrethroid insecticides (Starr et al. 2012) on motor activity in rats given single gavage doses of the mixtures. No statistically significant differences were found in observed motor activity values and predicted responses based on dose additivity and descriptions of the dose-response relationships for the individual components. A companion *in vitro* study assessing sodium influx in cerebrocortical neurons (presumably mediated by voltage-gated

sodium channels) found no statistically significant differences between observed effects from the 11-component mixture and effects predicted by a dose-additivity model (Cao et al. 2011). Based on results from these studies, the EPA (2011b) concluded that dose addition is a reasonable approach for estimating cumulative risk of exposures to mixtures of pyrethroids, noting several areas of uncertainty associated with this conclusion including whether dose additivity would predict responses to mixtures with different mixing ratios of components or by different exposure routes and duration.

- Statistically significant, greater-than-dose-additive effects at high doses and no significant deviation from dose additivity at low doses were observed on serum total thyroxine levels in young female rats given four daily gavage doses of an 18-component mixture of polyhalogenated aromatic hydrocarbons (2 dioxins, 4 dibenzofurans, and 12 PCBs, including dioxin-like and non-dioxin-like PCBs) (Crofton et al. 2005). The mixing ratio of the components was based on ratios of these chemicals found in breast milk, fish, and other sources of human exposure, and the mixture was given to rats at six dose levels ranging from approximately background levels to 100-fold greater than human background levels. The study included six to nine dose groups for each component to adequately characterize individual dose-response relationships. The statistical analysis used methods described by Gennings et al. (2002, 2004) and the definition of additivity described by Berenbaum (1985). Predicted responses based on additivity were about 2–3-fold less than observed responses at the three highest dose levels of the mixture, indicating a dose-dependent, greater-than-dose additive joint toxic action of relatively small magnitude.

- In several studies examining male reproductive system developmental end points (e.g., anogenital distance, nipple retention, testosterone production, other reproductive tissue malformations) in offspring of rats orally exposed to mixtures of chemicals that variably produce anti-androgenic effects via different mechanisms, dose-additive models provided adequate predictions of observed effects for most of the studies. The studied mixtures included vinclozolin, flutamide, and procymidone (Hass et al. 2007); diethylhexyl phthalate, vinclozolin, prochloraz, and fiansteride (Christiansen et al. 2009); di(*n*)butyl phthalate and diethylhexyl phthalate (Howdeshell et al. 2007); butyl benzyl phthalate, diethylhexyl phthalate, di(*n*)butyl phthalate, diisobutyl phthalate, and dipentyl phthalate (Howdeshell et al. 2008); and vinclozolin, procymidone, prochloraz, linuron, butyl benzyl phthalate, diethylhexyl phthalate, and di(*n)*butyl phthalate (Rider et al. 2008). Based on the results in these studies, the National Research Council (NRC) report, *Phthalates and Cumulative Risk Assessment: Tasks Ahead* (NRC 2008), recommended

that cumulative risk assessments should be conducted for phthalates producing common adverse outcomes on the developing male reproductive system using a dose-additive approach, regardless of mechanisms of action. Borgert et al. (2012) presented a critical evaluation of this recommendation noting several areas of uncertainty including limitations of the supporting study designs and analyses (e.g., each of the studies only looked at one mixing ratio of the components), extrapolations from relatively high exposure levels used in the rat studies to lower exposure levels expected to be experienced by humans, and evidence that humans may be less sensitive than rats to anti-androgenic chemicals, and commenting that a dose-additive/common adverse outcome approach to cumulative risk for phthalates and other anti-androgenic agents should only be used as a coarse, screening-level assessment.

- The TEF approach to assessing risks from mixtures of chlorinated dibenzo dioxins (CDDs) and related compounds is based on the assumption of dose additivity (see Section 3.3.5 for more details of this approach). Results from *in vivo* studies of animals exposed to defined mixtures of dioxins and dioxin-like compounds indicated that World Health Organization (WHO) recommended TEF values (Van den Berg et al. 2006) predicted mixture toxicities within a factor of about 2 or less (Fattore et al. 2000; Gao et al. 1999; Hamm et al. 2003; Walker et al. 2005), providing evidence that the dose additivity assumption in the TEF approach for dioxins and dioxin-like compounds is useful.

Results from in vitro Studies. Results from adequately designed *in vitro* studies of several end points (e.g., androgen receptor (AR) antagonism, estrogen receptor (ER) activation, ER-mediated cell proliferation, several genotoxicity end points) in cultured cells exposed to synthetic mixtures of up to 20–30 chemicals provide similar evidence that deviations from concentration addition (i.e., dose addition), when found, were small from a risk assessment perspective (mostly <5-fold deviation).

Androgen Receptor Antagonism Studies

- Concentration addition provided reasonable predictions of anti-androgenic activity (AR antagonism) in cultured human breast cells (MDA-kb2) exposed to mixtures of 8 pesticides showing only AR antagonism (Orton et al. 2012) and 17 AR antagonists with varying structural features (Ermler et al. 2011). The reporter gene assay used in these studies measured luciferase induction after AR activation by binding of an AR agonist (alpha-dihydrotestosterone, DHT); AR antagonism was measured in terms of suppression of DHT-induced luciferase-mediated

luminescence. The eight pesticides with only AR antagonism were fludioxonil, fenhexamid, ortho-phenyl phenol, imazalil, tebucoanzole, diethomorph, methiocarb, and primiphos-methyl. The 17 AR antagonists included several parabens (e.g., n-butyl paraben), UV-filter substances, benzo[a]pyrene, antioxidants (e.g., butylated hydroxytoluol), perfluorinated compounds, polybrominated and polychlorinated biphenyl ethers, and bisphenol A.

- Statistically significant deviations from concentration addition predictions were observed with mixtures of 5 pesticides, each showing both AR antagonism and agonism activities (cypronil, pyrimethanil, viclosolin, chlorpropham, and linuron tested at two mixing ratios) or mixtures of 13 pesticides (8 AR antagonists only and 5 with mixed antagonism and agonism activities tested at four mixing ratios), but these deviations were generally not large (Orton et al. 2012). Values of predicted ICs (IC_{10} or IC_{50}) for anti-androgenicity that were statistically significantly different from observed values (n=6) were mostly within 2-fold of observed values: five were lower than observed values but within 2-fold and one predicted value was greater than the observed value by about 6-fold (Orton et al. 2012).

- In another study with the same reporter gene assay, MDA-kb2 cells were exposed to mixtures of 30 AR antagonists from various classes of chemicals at three mixing ratios (Orton et al. 2014). Chemicals included pesticides, antioxidants, parabens, UV-filters, synthetic musks, bisphenol A, benzo[a]pyrene, perfluorooctane sulfonate, and pentabromodiphenyl ether. IC values predicted by concentration addition were slightly lower than observed values (within 2-fold) at all mixing ratios, whereas IC values predicted by independent action were greater than observed values by 2–4-fold (Orton et al. 2014).

- Concentration addition provided reasonable predictions of AR antagonism in an AR-reporter gene assay with Chinese hamster ovary CHO-K1 cells exposed to an equimolar mixture of two azole fungicides and one dithiocarbamate fungicide (biteranol, propiconazole, and mancozeb), but underestimated observed responses to an equimolar mixture of one triazine herbicide, two azole fungicides, one pyrethroid insecticide, and one organophosphate insecticide (terbuthylazine, biteranol, propiconazole, cypermethrin, and malathion) (Kjeldsen et al. 2013). For the five-component mixture, concentration addition predicted IC_{70}, IC_{80}, and IC_{90} values that were about 3-, 4-, and 9-fold higher than observed values (indicating greater-than-additive joint action).

- Another study reported that concentration addition adequately predicted the AR-antagonistic response in transfected CHO-K1 cells exposed to an equimolar mixture of five dissimilarly acting pesticides, deltamethrin (a pyrethroid insecticide), methiocarb (a carbamate insecticide), prochloraz (an azole fungicide), tribenuron-methyl (a sulfonylurea herbicide), and simazine (a triazine herbicide) (Birkhøj et al. 2004).

ER Activation or ER-Mediated Cell Proliferation Studies

- Concentration addition provided reasonable predictions of ER activation, with some reports of small deviations from additivity, in reporter gene assays of transfected mammalian cells or yeast cells exposed to mixtures of up to 3–17 estrogenic chemicals (Charles et al. 2002a, 2002b, 2007; Evans et al. 2012; Le Page et al. 2006; Payne et al. 2000; Rajapakse et al. 2002; Silva et al. 2002), as well as in cell proliferation assays in ER-competent MCF-7 human breast cancer cells exposed to mixtures of up to 17 estrogenic chemicals (Evans et al. 2012; Payne et al. 2001; Rajapakse et al. 2004; Silva et al. 2011; van Meeuwen et al. 2007). Results from a sample of these studies follows.

    - ER activation in transfected human MCF-7 breast cancer cells exposed to a fixed-ratio mixture of six synthetic chemicals with estrogenic activity (methoxychlor, o,p-DDT, octylphenol, bisphenol A, β-hexachlorocyclohexane, and 2,3-bis-(4-hydoxyphenyl)-propionitrile) was less than additive across a range of concentrations, but the magnitudes of deviation from concentration addition at each of the tested concentrations were <3-fold (Charles et al. 2007). In a companion *in vivo* immature rat uterotrophic assay, observed uterine weight responses to a mixture of these chemicals were statistically consistent with dose addition (Charles et al. 2007).

    - Responses to mixtures of 13–17 estrogenic chemicals at various mixing ratios were generally consistent with concentration addition in an ER reporter gene assay with human T47D-KBluc breast cancer cells and in a cell proliferation assay with MCF-7 cells (Evans et al. 2012). Low concentrations in the nanomolar range of a mixture of 16 chemicals (each component with minimal estrogenicity alone) did not affect the cell proliferative response of MCF-7 cells to a 14-component mixture of estrogenic chemicals, but inhibited the response at concentrations in the micromolar range.

- Cell proliferation responses in MCF-7 cells exposed to mixtures of six phytoestrogens (coumestrol, genistein, naringenin, catechin, epicatechin, and quercetin), six synthetic chemicals with estrogenic activity (4-nonylphenol, octylphenol, β-hexachlorohexane, bisphenol A, methoxychlor, and dibutyl phthalate), or a combination of both mixtures showed no statistically significant deviations from concentration addition predictions (van Meeuwen et al. 2007).

- Mixtures of 8, 10, 11, or 16 estrogenic chemicals produced cell proliferative responses in MCF-7 cells that were: (1) adequately predicted by concentration addition for the 8-component mixture and (2) overestimated by concentration addition for the 10-, 11-, and 16-component mixtures, indicative of less-than-additive joint action (Silva et al. 2011). For the latter three mixtures, observed effective concentrations were greater than predicted values by factors ranging from about 1.5- to 5-fold (Silva et al. 2011).

Genotoxic End Point Studies

- Concentration addition adequately explained effects on micronuclei formation in CHO-K1 cells exposed to a mixture of seven aneugenic benzimidazole pesticides, which act by a similar mechanism: inhibition of microtubule formation by binding to β-tubulin monomers at the colchicine-binding site (Ermler et al. 2013). In a subsequent study of mixtures of four to five chemicals inducing micronuclei by different mechanisms (aneugens and clastogens), the observed micronuclei responses to the mixtures were larger than responses predicted by independent action (i.e., response addition), but less than those predicted by concentration addition (Ermler et al. 2014).

- Studies with cultured cells of mouse lymphoma cells (L5178Y) and human cell lines (TK 6 and WTK1) exposed to gamma-ionizing radiation from ^{137}Cs and ethyl methanesulfonate, showed micronuclei induction responses in the human cell lines that were consistent with concentration addition, but greater-than-additive action (40% supra-additive effect) in L5178Y cells (Lutz et al. 2002).

- Concentration addition predictions were not significantly different from observed mutation responses in bacteria (*Salmonella* Ames assay) to a mixture of three PAHs (benzo[a]pyrene, benz[a]anthracene, and dibenz[a,c]anthracene) (Lutz et al. 2002).

- Concentration addition predictions were mostly greater than observed mutation responses in several strains of *Salmonella* to complex mixtures that were nonpolar fractions of extracts from 10 soils contaminated with complex PAH-containing mixtures (Lemieux et al. 2008). The soils were from Swedish sites at which creosote wood preservation (n=7), coke production (n=1), or gas manufacturing (n=2) had occurred for many years. The concentration addition predictions were made based on individual relative potencies for eight nonsubstituted, homocyclic PAHs showing positive activity in the assay conducted with three *Salmonella typhimurium* strains. (Sixteen PAHs identified by the EPA as Priority PAHs were tested, and 8 showed positive results.) Sixty-eight percent of predicted values were statistically significantly greater than observed values; 94% of these values were within about 4-fold of observed values, and one was greater by about 8-fold (Lemieux et al. 2008). Twelve percent of predicted values were not significantly different from observed values, and 20% were less than corresponding observed values (Lemieux et al. 2008). In a subsequent study of mutagenic activities of nonpolar fractions of extracts of the same soils in an *in vitro* version of the LacZ transgenic rodent mutation assay, predicted values of mutagenic activity based on dose addition of individual mutagenic PAHs were within 2-fold of observed values for 9 out of 10 of the nonpolar fractions of soil extracts (Lemieux et al. 2015).

- In a tiered experimental design, deviations from response additivity, both greater-than-additive and less-than-additive, were detected in studies of the effects of mixtures of five mycotoxins with different mechanisms of action on inhibition of DNA synthesis in mouse fibroblast L929 cells (Tajima et al. 2002).

Summary of Evidence Related to Dose Additivity as a Default Assumption for Component-Based Approaches to Assessing Noncancer Health Impacts. Based on the above *in vivo* and *in vitro* evidence, the dose-additivity assumption appears to be a reasonable *default* assumption for screening-level assessments of mixtures of chemicals with similar effects or the same target organ. Results from adequately designed studies of various end points affected by defined mixtures of various classes of chemicals showed that: (1) dose additivity often provided adequate descriptions of the mixture responses and (2) positive and negative deviations from dose additivity were small from a risk assessment perspective (generally <5-fold). In addition, results from a few studies of end points in cells exposed to mixtures of components with differing MOAs indicated that observed responses were intermediate between the values predicted by concentration addition (i.e., dose addition) and response addition (independent action) (Ermler et al. 2014; Orton et al. 2014).

In support of these conclusions, an independent analysis of research studies (published between 1990 and 2008) reporting "synergy" at dose levels close to PODs for individual mixture components (i.e., "low" doses) identified only 11 out of 90 studies reporting "synergy" in which the magnitude of "synergy" was calculated (Boobis et al. 2011). Among those 11 studies, 6 studies used comparable methods to indicate that the magnitude of synergy was small from a risk assessment perspective, ranging from about 1.9- to 3.5-fold greater than additivity (Boobis et al. 2011). Three of the six studies (Crofton et al. 2005; Moser et al. 2005, 2006) were described in the bulleted items above.

Although the research results reviewed herein provide support for the use of dose-additivity as a default assumption in component-based approaches, they also provide evidence for cases of deviations from additivity. In addition, much of the evidence is based on short-term exposures. Further research may help to confirm or refute the validity of this assumption, in particular for chronic exposure scenarios and for early life exposures with possible later life health outcomes. As such, environmental scientists should be aware that currently recommended approaches to assess health impacts from combined exposure to multiple agents (as discussed in Chapter 2) are practical tools which could overestimate or underestimate actual health impacts.

3.3.2. Hazard Index Approach

The hazard index approach uses the assumption of dose additivity to assess the noncancer health effects of a mixture from the data on the components. The approach, or some modification of it, is used or recommended by a number of agencies (including ATSDR), especially as a tool for screening-level assessments (see Chapter 4 of this document: ACGIH 2015; CPSC 2014; DEPA 2009; EC 2012; EFSA 2013; EPA 1986, 1989a, 2000, 2011c; Feron et al. 2004; Meek 2013; Meek et al. 2011; Mumtaz et al. 1994a, 1997; NAS 1974; Norwegian Scientific Committee for Food Safety 2013; NRC 1989; OSHA 1993, 2001; Yu et al. 2010, 2013). In this approach, exposures or doses for the various components of a mixture of concern are compared with a defined level of exposure generally regarded as acceptable or safe (public health guidance value) by the agency performing the assessment. The defined levels could be ATSDR MRLs, EPA RfDs or RfCs, ACGIH threshold limit values (TLVs), or Occupational Safety and Health Administration (OSHA) permissible exposure limits (PELs). The general equation for the hazard index (*HI*) is:

$$HI = \frac{E_1}{DL_1} + \frac{E_2}{DL_2} + \text{.....} + \frac{E_n}{DL_n} \quad (1)$$

In Equation 1, E_1 is the level of exposure to the first chemical in the mixture and DL_1 is some defined level of "safe" exposure to the first chemical, E_2 and DL_2 are the corresponding levels for chemical 2, and the summation can extend to any number of chemicals, signified by the *n*. Each chemical-specific ratio (e.g., E_1 / DL_1) is called a hazard quotient (*HQ*). Therefore, the hazard index can be expressed as the sum of the HQs:

$$HI = \sum_{i=1}^{n} HQ_i \qquad (2)$$

When the HQ for a single chemical exceeds unity, concern for the potential hazard of the chemical increases. Similarly, when the hazard index for a mixture exceeds unity, concern for the potential hazard of the mixture increases.

Separate hazard indices are usually estimated for each exposure pathway and exposure duration of concern. For a given duration, hazard indices can be summed across pertinent exposure pathways that affect the same receptor population, giving an indication of cumulative impact or risk from components in the mixture.

The obvious advantage of this method is its simplicity. Because it is based on the assumption of dose additivity, the hazard index method is most appropriately applied to components that cause the same effect by the same mechanism or mode of action. In practice, it may be applied to components with different target organs as a screening measure. The method is also frequently applied to components with the same critical target organ or critical effect (effect that is the basis for the MRL, RfD, or other health guideline), without regard to mechanism or mode of action. For Superfund risk assessments, strong evidence is required to indicate that two compounds producing adverse effects on the same organ system, although by different mechanisms, should not be treated as dose additive (EPA 1989a, 2000). See also the discussion in Section 3.3.1.2 (*Evidence to Support or Refute the Use of Default Dose-Additivity Approaches*).

The ATSDR (2005a) *Public Health Assessment Guidance Manual* notes that there is no evidence of additive toxicity from exposure to components of a mixture when individual chemicals are administered well below their individual apparent toxicity thresholds (Seed et al. 1995; Wade et al. 2002). It recommends that when the site-specific hazard index is <1.0, "it is highly unlikely that significant

additive or toxic interactions would occur, so no further evaluation is necessary." When the hazard index exceeds 1.0, further evaluation is recommended, specifically that the assessor should compare the estimated exposure level for each component to the NOAEL on which the MRL is based. These comparisons represent POD HQs, as opposed to "guidance value" HQs. ATSDR (2005a) recommends that if exposure to one or more of the components is within 1 order of magnitude of the guidance value NOAEL (0.1xNOAEL), the assessor should conduct more in-depth analysis such as calculating hazard indices for components with common adverse effects (i.e., target organ or tissue) or common adverse effects via a common MOA and qualitatively evaluating information on possible interactions among components. Furthermore, ATSDR (2005a) recommends that if estimated exposure levels of all components are less than one-tenth of the respective PODs (i.e., NOAEL, LOAEL, or BMDL), then significant additive or interactive effects are unlikely and further in-depth evaluation of potential health impacts from exposures to multiple chemicals at the site is unnecessary. However, ATSDR (2005a) also noted that assessors may proceed with further evaluations in some instances, such as when several or more components in the mixture produce the same health effect either by different or common MOAs, when there are concerns for sensitive populations in the community, when the PODs for the MRLs are uncertain, or for other reasons (see Chapter 2 of this document for more specific and extensive discussion of assessing health impacts from multiple agents).

The hazard index method does not take into account interactions among the components of the mixture, but methods to modify the method by incorporating data on possible interactions (deviations from additivity) among components are described in Section 3.3.3.

Additional information on the hazard index method is provided in EPA (1986, 1989a, 2000).

3.3.3. Target-organ Toxicity Dose (TTD) Modification to Hazard Index Approach

The TTD approach, which is a refinement of the hazard index approach, was devised in order to accommodate the assessment of mixtures whose components do not all have the same critical effect (i.e., the most sensitive effect providing the basis of the public health guidance value), but may produce toxic effects in common target organs dependent on exposure level. It takes into account the reality that most components of contaminated-site-related mixtures affect other target organs at doses higher than those that cause the critical effect of the guidance value. These other effects may vary from component to component and may be important in assessing the health effects of the mixture. EPA (1989a) suggested that separate hazard indices be estimated for all end points of concern, and that the RfD be used not only

in generating HQs for the critical effect of a component, but also in estimating HQs for effects that occur at higher exposure levels. As acknowledged by EPA (1989a) and demonstrated by Mumtaz et al. (1994a, 1997), this practice may overestimate the hazard for effects occurring at exposure levels higher than those associated with the critical effect. The use of TTDs was therefore suggested (Mumtaz and Colman 1993; Mumtaz et al. 1997). TTDs are developed for the chemicals that affect an end point at a dose higher than that for the critical effect for the same chemical. A TTD for each end point of concern is calculated using appropriate MRL (or RfD) methodology, and then used in estimating the end-point-specific HQs and hazard indices. The MRL (or RfD) is used for the critical effect for each chemical and the TTD is used for the other end points of concern for the chemical. When any of the end-point-specific hazard indices exceeds unity, concern for the potential hazard of the mixture increases.

The derivation of TTDs for use in assessment of the joint toxic action of chemical mixtures is analogous to the derivation of MRLs, and should follow the applicable portions of ATSDR MRL guidance (ATSDR 1996). TTDs are based on the other major characteristic effects of a chemical, which are known to occur at the same or higher exposure levels as the critical effects. Like the derivation of an MRL, the derivation of a TTD is not recommended for an end point that is affected only at the relatively high levels of exposure associated with severe effects. Because the purpose of TTD derivation is to support the estimation of end-point-specific hazard indices (Mumtaz et al. 1994a, 1997), TTD derivations should be performed for end points that are common to more than one component of a given mixture. In addition, end points identified as concerns in populations exposed to the mixture should be considered.

Like MRLs (or RfDs), TTDs are specific for route and exposure period. The TTD should be based on the highest NOAEL that does not exceed a LOAEL for the particular end point, as determined from the information in toxicological profiles, including the Levels of Significant Exposure tables. If such a NOAEL is not available, the TTD would be based on the lowest LOAEL for that end point; PODs for TTDs should be from a representative, high-quality study involving the route and exposure duration of concern. When data for the exposure duration of concern are not available, a TTD derived for one duration may sometimes be applicable for other duration(s) of the same route, if supported by the overall database. An additional uncertainty factor may be applied to account for uncertainty associated with duration extrapolation, based on scientific judgment. Dose adjustments and interspecies, intraspecies, and LOAEL-to-NOAEL extrapolation (i.e., uncertainty factors) should be performed and explained as for an MRL. When suitable data are available, and when appropriate, TTDs can also be derived using BMD PODs (Crump 1984, 1995; EPA 2012a; Gaylor et al. 1998) to define the BMDL, which is used in place of a NOAEL as the basis for TTD derivation, similar to the procedure for MRL derivation.

An illustrative example follows of the application of the TTD-modification of the hazard index to a hypothetical site-specific mixture of chemicals 1, 2, 3, and 4 to which intermediate-duration oral exposure is of concern. The intermediate oral MRLs are based on critical hepatic effects for chemicals 1 and 2, and critical renal and critical developmental effects, respectively, for chemicals 3 and 4. Each of these end points also is affected by at least one other mixture component for which it is not the critical effect. Other major effects in common for two or more of these chemicals for this route and duration include neurological and developmental effects. In addition, chemical 1 causes immunological effects and chemical 4 causes endocrine (adrenal) effects during intermediate oral exposure. At levels of exposure that cause high mortality, chemical 1 also causes hematological effects in rats. This information is summarized in Table 3.

Table 3. End Points Affected by Chemicals 1, 2, 3, and 4

	Affected by:			
End point	Chemical 1	Chemical 2	Chemical 3	Chemical 4
Hematological	With mortality	No	No	No
Hepatic	Yes—MRL	Yes—MRL	No	Yes
Renal	Yes	No	Yes—MRL	Yes
Endocrine (adrenal)	No	No	No	Yes
Immunological	Yes	No	No	No
Neurological	Yes	Yes	Yes	No
Developmental	Yes	Yes	Yes	Yes—MRL

MRL = Minimal Risk Level

The end points of concern chosen for TTD derivation, based on the critical effects of the chemicals and on other major effects in common for this set of chemicals, are hepatic, renal, neurological, and developmental effects. These end points are shown in bold italicized print in the table. Since adrenal and immunological effects each are caused by only one chemical, and are not the critical effects for any of the components of the mixture, the estimation of end-point-specific hazard indices is not needed for these end points, and TTDs are accordingly not developed. For a different mixture of chemicals that included chemical #1, the immunological end point may warrant TTD derivation if at least one other chemical in the mixture also causes this effect. Similar reasoning would apply for chemical #4 and adrenal effects. The hematological effects are not a suitable basis for TTD derivation for chemical #1 not only because they are caused by only one chemical, but also because they occurred only at levels of exposure that caused significant mortality.

For the purposes of illustration, a TTD for renal effects will be derived for chemical #1. The intermediate oral MRL for chemical #1 is 0.15 mg/kg/day based on a NOAEL of 15 mg/kg/day for hepatic effects in experimental animals given the chemical orally for an intermediate duration. The NOAEL was divided by an uncertainty factor of 100 (10 for interspecies and 10 for intraspecies variability) to estimate the MRL. The LOAEL for hepatic effects in the same study was 30 mg/kg/day. The NOAEL and LOAEL values for renal effects in this study were 30 and 45 mg/kg/day, respectively, and were the most reliable data for this effect. In addition, the NOAEL was the highest NOAEL for this effect. A TTD_{RENAL} of 0.3 mg/kg/day for chemical #1 is derived by dividing the $NOAEL_{RENAL}$ of 30 mg/kg/day by an uncertainty factor of 100 (10 for interspecies and 10 for intraspecies variability). Derivation of TTDs for the other effects would proceed in a similar manner.

Following derivation of the TTDs, end-point-specific hazard indices are calculated as follows:

$$
\begin{aligned}
(a) \quad & HI_{HEPATIC} = \frac{E_1}{MRL_1} + \frac{E_2}{MRL_2} + \frac{E_4}{TTD_{4\,HEPATIC}} \\
(b) \quad & HI_{RENAL} = \frac{E_1}{TTD_{1\,RENAL}} + \frac{E_3}{MRL_3} + \frac{E_4}{TTD_{4\,RENAL}} \\
(c) \quad & HI_{NEURO} = \frac{E_1}{TTD_{1\,NEURO}} + \frac{E_2}{TTD_{2\,NEURO}} + \frac{E_3}{TTD_{3\,NEURO}} \\
(d) \quad & HI_{DEV} = \frac{E_1}{TTD_{1\,DEV}} + \frac{E_2}{TTD_{2\,DEV}} + \frac{E_3}{TTD_{3\,DEV}} + \frac{E_4}{MRL_4}
\end{aligned}
\qquad (3)
$$

where $HI_{ENDPOINT}$ is the hazard index for indicated end point (*HEPATIC*, *RENAL*, *NEURO* [neurological], *DEV* [developmental]), E_i is the exposure for the i^{th} chemical (1, 2, 3, or 4 in the above example), MRL_i is the MRL for the i^{th} chemical, and TTD_i is the TTD for the i^{th} chemical for the indicated end point. (If an MRL is not available, a suitable RfD can be used.) Although developmental toxicity is the critical effect for only one of the four chemicals, all four produce the effect, and it is conceivable that it may be a sensitive effect for the mixture. Neurological effects are not the critical effect for any of the chemicals, but three of the chemicals cause this effect at equivalent or higher exposure levels than associated with the critical effect. Thus, use of the TTD modification of the hazard index for mixtures of chemicals that do not have the same critical effect may increase the understanding of the potential impact of the mixture on public health. Additional information regarding this method is provided by Mumtaz et al. (1994a, 1997).

The development of TTDs can be analytically intensive. TTDs have been developed for a variety of chemicals in a pilot study (Mumtaz et al. 1997) and in a number of ATSDR interaction profiles (Pohl and Abadin 2008; Pohl et al. 2003, 2004, 2009; see www.atdr.cdc.gov/interaction profiles). The derivations in the interaction profiles are subjected to a review process that is similar to that for MRLs. Currently, ATSDR Toxicological Profiles only present MRLs for subject chemicals and do not present TTDs.

3.3.4. Weight-of-Evidence (WOE) Modification to the Hazard Index Approach

As noted above, the hazard index approach does not incorporate information on interactions among components of the mixture. A WOE method proposed by Mumtaz and Durkin (1992) was the first systematic attempt to address this need. The method implemented and expanded on the suggestion made by the NRC (1989) that, in recognition of the difficulties of quantifying interactions, an uncertainty factor be used to account for interactions among components of a mixture. The method was designed to modify the hazard index to account for interactions, using the WOE for interactions among pairs of mixture components. Although subsequent experience with the algorithm used to generate the interactions hazard index has revealed that it does not account for changes in proportions of mixture components in a reasonable manner, the method is useful qualitatively for predicting whether a hazard may be greater or less than indicated by the hazard index.

The method evaluates data relevant to joint action for each possible pair of chemicals in the mixture in order to make qualitative binary WOE (BINWOE) determinations for the effect of each chemical on the toxicity of every other chemical. Two BINWOEs are needed for each pair: one for the effect of chemical A on the toxicity of chemical B, and another for the effect of chemical B on the toxicity of chemical A. The BINWOE determination is a classification that indicates the expected direction of an interaction (greater than additive, less than additive, additive, or indeterminate), and scores the data qualitatively, using an alphanumeric scheme that takes into account mechanistic understanding, toxicological significance, and relevance of the exposure duration, sequence, bioassay (*in vitro* versus *in vivo*), and route of exposure. The alphanumeric terms in the classification scheme can then be converted to a single numerical score, by multiplying the corresponding direction factor by the data quality weighting factor. Although earlier publications of the WOE method did not discuss the need for target organ consideration in BINWOE determinations (Mumtaz and Durkin 1992), experience in application of the WOE method, including preparation of the ATSDR interaction profiles and a study by Mumtaz et al. (1998), has indicated that the WOE evaluations should be target-organ specific.

The qualitative BINWOE classifications are shown in the left column of Table 4 and the direction factors and data quality weighting factors are shown in the far right column. An alphanumeric (qualitative) BINWOE classification of >II.B.2.a.i for the effect of one chemical on the toxicity of another thus corresponds to greater-than-additive interaction, mechanistic data on related chemicals, inferred toxicological significance, different duration or sequence, *in vivo* data, and anticipated route of exposure. The corresponding BINWOE score is +1(0.71)(0.71)(0.79)(1)(1)=+0.40.

Table 4. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions

Classification		Factor
Direction of Interaction		**Direction**
=	Additive	0
>	Greater than additive	+1
<	Less than additive	−1
?	Indeterminate	0
Quality of the Data		**Weighting**
Mechanistic Understanding		
I.	Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.	1.0
II.	Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur have not been well characterized for the chemicals of concern but structure-activity relationships, either quantitative or informal, can be used to infer the likely mechanisms(s) and the direction of the interaction.	0.71
III.	Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.	0.32
Toxicological Significance		
A.	The toxicological significance of the interaction has been directly demonstrated.	1.0
B.	The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.	0.71
C.	The toxicological significance of the interaction is unclear.	0.32
Modifiers		
1.	Anticipated exposure duration and sequence.	1.0
2.	Different exposure duration or sequence.	0.79
a.	*In vivo* data	1.0
b.	*In vitro* data	0.79
i.	Anticipated route of exposure	1.0
ii.	Different route of exposure	0.79

Weighting factor = product of weighting scores: maximum = 1.0, minimum = 0.05

BINWOE = direction factor x weighting factor: ranges from −1 through 0 to +1

Sources: Mumtaz and Durkin 1992; Mumtaz et al. 1994a

The qualitative WOE approach, focusing on application of the BINWOE scores to hazardous waste site assessment, was suggested by Mumtaz and Durkin (1992). This approach was recommended for a mixture where the scaled doses (HQs) for all of the components are similar, or toxicologically significant. The qualitative BINWOE scores for the components, if similar in direction, are the basis for a conclusion. For example, consider a mixture of four components, all present at toxicologically significant levels. The number of possible chemical pairs in a mixture of N components is $(N^2-N)/2$. Thus, this mixture of 4 components has 6 pairs of components and potentially 12 BINWOEs. Suppose nine of the BINWOEs are greater than additive (positive) with alphanumeric classifications indicating a relatively high degree of confidence, and the remaining three BINWOEs are additive (0), also with relatively high degrees of confidence. In this case, the WOE suggests that the mixture is likely to pose a greater hazard than that indicated by the hazard index.

A likely pattern of qualitative BINWOEs for a mixture is a mixed pattern (some greater-than-additive, some less-than-additive, and some additive BINWOEs). In this case, the qualitative WOE approach is extended to include conversion of the qualitative BINWOE scores to numerical scores, and summing the scores to give a combined score. If the combined BINWOE score is positive and significantly different from zero, then the WOE suggests that the mixture is likely to pose a greater hazard than indicated by the hazard index. Conversely, if the combined BINWOE score is negative and significantly different from zero, then the WOE suggests that the health hazard is unlikely to be greater than indicated by the hazard index. Professional judgment is used in the interpretation of the impact of the WOE on the hazard index.

Although the WOE method was developed for assessing interactions for noncarcinogenic effects, the qualitative WOE method is equally applicable to assessing interactions for carcinogenic effects.

The WOE method has undergone evaluation, and appeared to perform well qualitatively (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). The application of the method for deriving BINWOE classifications was considered consistent by expert toxicologists who reviewed the results of exercises in which several teams of toxicologists and risk assessors independently determined BINWOE classifications for the same pairs of chemicals (Mumtaz et al. 1994b). In tests of the WOE method to predict the toxicity of some simple chemical mixtures to animals, BINWOEs for three pairs of chemicals qualitatively predicted whether the results of animal studies would be less than additive, additive, or greater than additive (Mumtaz et al. 1998). Used with an exponential dose-response model and dose addition to model relative kidney weights, the quantitative WOE method closely predicted the observed dose-response in female rats for intermediate-duration oral exposure to a mixture of four nephrotoxic chemicals with similar MOAs

(Mumtaz et al. 1998). The observed dose-response was less than dose additive. The BINWOEs were focused on renal toxicity, and the uncertainty factor used in the algorithm was 10. The WOE method underestimated the relative liver weights in the same animals. The observed dose-response for relative liver weight was slightly greater than dose additive. Thus, the WOE method did not predict toxicity to a target organ that was different from the one for which the BINWOEs were derived. The WOE method slightly overpredicted the observed dose-response for relative kidney weight in male rats for a mixture of dissimilarly acting nephrotoxins (in female rats, the data variability was so great that the exponential model did not fit the *observed* responses) (Mumtaz et al. 1998). Although these results are suggestive, limitations of this test of the complete WOE method include the substantial variability in the responses of individual animals, small numbers of animals per group, testing of only two dose levels of the mixtures, and lack of rationale for using relative organ weight as an index of toxicity (several other indicators of renal and hepatic toxicity were monitored in the studies that provided the experimental data [Jonker et al. 1993, 1996]).

Possible applications of the qualitative BINWOE approach during refined Tier 3 analysis (see Section 2.4) include qualitative assessment that a hazard index is overprotective when evidence indicates that dose responses are less-than-additive or under-protective when evidence indicates that dose responses are greater-than-additive for any two (or more) components in the evaluated mixture.

A modification of the original WOE method was adopted as part of EPA's mixtures guidance (EPA 2000). This modification includes a slightly different classification scheme and a method of calculating an interactions-modified hazard index. The method encourages greater use of quantitative interaction data through the use of magnitude-of-interaction factors for each chemical pair. The classification scheme, while more integrated in nature, requires more judgment, and the type of quantitative interaction data required to estimate the magnitude factor is rarely available (see Boobis et al. 2011). The algorithm for this modification appears to handle changes in proportions of mixture components more reasonably than does the original algorithm, but additional evaluation with regard to predicting experimental results is desirable.

A basic assumption of both WOE methods is that interactive interference will not be significant. For example, if chemicals A and B interact in a certain way, the presence of chemical C will not cause the interaction to be substantially different. Thus, the assumption is that pairwise interactions will dominate in the mixture and will adequately represent all of the interactions.

Additional detail regarding both methods is provided in Appendix B.

3.3.5. Relative Potency Factor (RPF) Approaches (including Toxicity Equivalency Factor [TEF] Approaches)

RPF approaches are developed and used to evaluate mixtures of related chemicals that are assumed to be toxicologically similar, for cases in which dose-response data for one chemical in the chemical group (termed the index chemical or compound) are sufficient to derive a guidance value (e.g., an MRL, RfD, or cancer slope factor), but dose-response information for the other chemicals is less complete (EPA 2000). RPF approaches require both exposure and toxicity data and scale exposure concentrations of the non-index chemicals relative to the potency of the index chemical using scaling factors (i.e., RPFs) based on a specific toxic effect, route of exposure, or duration of exposure. A TEF approach is a special type of RPF approach for a group of chemicals sufficiently well studied to have confidence that the scaling factors (i.e., TEFs) are applicable to all health endpoints, all routes of exposure, and all durations of exposure. Compared with a generalized RPF approach, a TEF approach is based on more high-quality and abundant mechanistic data yielding considerable certainty about the MOA leading to all shared toxic effects from members in the group (EPA 2000). In essence, a TEF may be developed if there is confidence that a single MOA or toxicity pathway is shared by all members of the group. The classic example of a TEF approach is the one developed for dioxins and dioxin-related compounds, which share a key event (binding to the aryl hydrocarbon receptor) leading to downstream adverse effects (see next paragraph).

The most widely accepted TEF approach is used with the CDDs and structurally related chemical classes such as the chlorinated dibenzo-*p*-furans (CDFs) and the coplanar PCBs that are expected to have a common mechanism of action in producing common adverse outcomes (Ahlborg et al. 1994; ATSDR 1998b; EPA 1989b, 1994, 2010b 2012b; Safe 1998; Van den Berg et al. 1998, 2006). This method estimates TEFs for the various congeners in the mixture based on the key assumptions that CDD and CDF congeners produce nonneoplastic and neoplastic effects through a common receptor-mediated MOA (aryl hydrocarbon receptor), and act in a dose-additive manner. The TEF approach uses data from *in vitro* and *in vivo* studies comparing the potency of individual congeners to produce toxic or biological effects, with that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the best-studied of this chemical class. Relative potencies are calculated from these studies as the ratio of the EC_{50} for a congener to the EC_{50} for 2,3,7,8-TCDD (Van den Berg et al. 2006). 2,3,7,8-TCDD is assigned a TEF of one, and TEF values for the other congeners are determined by an evaluation of the range of relative potency estimates from the available studies (Van den Berg et al. 2006). The most recent consensus values for TEFs for CDDs, CDFs, and dioxin-like PCBs were determined by an expert panel convened by the WHO (Van den Berg et

al. 2006). A 2011 expert consultation evaluated the possible inclusion of brominated analogues of the dioxin-like compounds in the WHO TEF scheme, and recommended the use of similar interim TEF values for brominated and chlorinated congeners for human health risk assessment (Van den Berg et al. 2013).

To assess exposure to a specific mixture of CDDs and dioxin-like compounds, the concentrations of congeners present in a mixture in environmental media are determined and multiplied by their TEF values and then summed to give the total 2,3,7,8-TCDD toxic equivalents (TEQs) of the mixture:

$$TEQs = \sum_{i=1}^{n} C_i \times TEF_i \quad (4)$$

where C_i is the concentration and TEF_i is the TEF for the i^{th} component of the mixture. The TEQ thus represents the concentrations of all of the components as an equivalent concentration of the index chemical, 2,3,7,8-TCDD, based on the assumption of dose additivity. The TEQs are used in exposure models to estimate intakes for target populations and specific exposure scenarios. An index of hazard for noncancer health effects is estimated by comparing (via HQs) the TEQ intake with the appropriate MRL for 2,3,7,8-TCDD or other health-based criteria (e.g., the EPA RfD for 2,3,7,8-TCDD) (ATSDR 1998b, 2008b; De Rosa et al. 1997a, 1997b, 1997c, 1998; EPA 2010b; Mumtaz and Hertzberg 1993; Pohl et al. 1995). If the ratio of the TEQs to the guidance value for 2,3,7,8-TCDD is >1, there is concern for increased risk of hazard; values <1 do not merit concern for increased risk. For cancer risk assessment, an estimate of cancer risk is obtained by multiplying the TEQ (in appropriate units of mg/kg/day or mg/m^3) by a cancer slope factor or unit risk for 2,3,7,8-TCDD (EPA 1994, 1996; Mumtaz and Hertzberg 1993).

This TEF approach is considered suitable for the assessment of health effects of dioxin-like compounds that are mediated through the aryl hydrocarbon receptor, but is not applicable for those that are not (ATSDR 1998b; Van den Berg et al. 2006). Aryl hydrocarbon receptor mediation is thought to be a key event in the mechanism of action for effects produced by this class of chemicals including carcinogenicity, immunotoxicity, and developmental and reproductive toxicity (the basis for oral MRLs and the EPA IRIS [2012] RfD for 2,3,7,8-TCDD) (ATSDR 1998b; EPA 2010b, 2012b; Van den Berg et al. 2006). Limitations to this method are that: (1) some of the nondioxin-like PCB congeners have been shown to inhibit or enhance responses to 2,3,7,8-TCDD, depending on dose and assay system (Birnbaum

and DeVito 1995; Pohl and Holler 1995; Safe 1998); (2) some PCB congeners with many relative potency studies have a very broad range of relative potency estimates (Safe 1998; Van den Berg et al. 2006); and (3) a slope factor for 2,3,7,8-TCDD is not available on the EPA IRIS (2012). The TEF approach continues to evolve and undergo additional testing and validation. ATSDR considers the approach less suitable for PCBs, and has derived MRLs for PCBs (ATSDR 2000b). ATSDR uses the TEF method as a tool for assessing health effects of dioxin and dioxin-like compounds (primarily CDDs and CDFs) in soil (ATSDR 1998b, 2008b; De Rosa et al. 1997a, 1997b, 1997c, 1998). The most recent consensus TEF values presented by WHO (Van den Berg et al. 2006) are part of the methods used by ATSDR (2008b) and EPA (2010b) to assess human health risks of mixtures with dioxins and dioxin-like compounds. Results from *in vivo* studies of defined mixtures of dioxins and dioxin-like compounds indicated that WHO TEF values predicted mixture toxicity within a factor of ≤2 (Fattore et al. 2000; Gao et al. 1999; Hamm et al. 2003; Walker et al. 2005), providing evidence that the dose-additivity assumption in the TEF approach for dioxins and dioxin-like compounds is useful. Evidence that bioavailability of CDDs and CDFs can be limited in soils, and the fact that most TEFs for CDDs, CDFs, and dioxin-like PCBs are based on data from studies of laboratory animals fed test compounds in food, has led to recommendations that adjustments be made to account for decreased bioavailability in soil, when risks are assessed from exposures to dioxins and dioxin-like compounds in soil (EPA 2007a, 2010a; Van den Berg et al. 2006).

An RPF approach has been developed for nonsubstituted PAHs that have been classified as B2 carcinogens by EPA (ATSDR 1995b; EPA 1993). The RPFs (termed estimated order of potency by EPA [1993]) were estimated on the basis of potency relative to that of benzo[a]pyrene in mouse skin tumor studies. RPFs for a wider number of individual nonsubstituted PAHs (up to 24) have been developed by a number of groups (see Jarvis et al. [2014] for review). Benzo[a]pyrene is the best-studied member of this class and has a cancer OSF available on IRIS (1998a). Similar to the TEF approach, the concentrations of PAHs with RPFs are first determined in environmental media. Exposure models are then used to estimate oral intakes of each PAH for target populations, and individual PAH intakes are converted to benzo[a]pyrene equivalents with the appropriate RPF. The benzo[a]pyrene equivalents are then summed and multiplied by the benzo[a]pyrene cancer slope factor to obtain an estimate of the cancer risk in the target population from the carcinogenic PAHs in the mixture. The mechanistic underpinnings of the RPF approach for the PAHs are less compliant than CDDs with the assumption of a single common, mechanism-of-action key event, as multiple mechanisms are likely involved for different PAHs (see Boström et al. 2002; Jarvis et al. 2014). In addition, some of the same issues noted for the application of the TEF approach for CDDs also are issues for the use of the RPF approach for PAHs,

including evidence for greater-than-additive and less-than-additive interactions among binary and more complex mixtures of PAHs and the wide range in published RPF values for many individual PAHs (see Jarvis et al. [2014] for review). Several reports have indicated that the RPF approach may be inadequate for predicting carcinogenic responses in mouse-skin tumor-initiation studies of complex PAH-containing mixtures or certain PAHs (e.g., dibenzo[def,p]chrysene) having mechanisms of action different from those of benzo[a]pyrene (Courter et al. 2008; Siddens et al. 2012; Tilton et al. 2015). Other reports suggest that the mutagenic activities of extracts of PAH-contaminated soil are inadequately predicted by dose addition and RPFs due to competing factors of: (1) possible less-than-additive, metabolism-related, interactions among components and (2) contributions from non-identified mutagenic components in the extracts (Lemieux et al. 2015, 2008). However, in these studies, mutagenic activities predicted by dose addition of PAH components were mostly within 2–4-fold of observed mutagenic activities of nonpolar fractions of extracts of 10 soils contaminated with complex PAH-containing mixtures (Lemieux et al. 2015, 2008).

EPA OPP has developed cumulative risk assessments for classes of pesticides whose members produce common effects by a common mechanism using an RPF approach coupled with a POD/Margin of Exposure (MOE) approach as an index of risk (EPA 2002b). The EPA OPP approach for cumulative risk assessments involves: (1) determination of whether or not a group of structurally related pesticides produces a common effect by a common mechanism; (2) selection of an index chemical and determination of RPFs for members of the group; (3) determination of concentrations of member chemicals in foods and environmental media; (4) estimation of intakes for target population for multiple exposure pathways using exposure models; and (5) assessment of risks for target populations using a POD/MOE hazard indicator method when appropriate data are available (EPA 2002b).

The OPP cumulative risk assessments each began with a WOE evaluation identifying a group of chemicals that produce a common effect by a common mechanism (EPA 2002b). Using this type of evaluation, OPP determined that there was sufficient evidence for a common effect by a common mechanism for five pesticide classes (three insecticide classes and two herbicide classes) including organophosphates (EPA 2006b), N-methyl carbamates (EPA 2007b), pyrethrins/pyrethroids (EPA 2011b), triazines (EPA 2006d), and chloroacetanillides (EPA 2006c), but insufficient evidence for members of the thiocarbamate class (EPA 2001a) or members of the dithiocarbamate class (EPA 2001b).

The index chemical for the common assessment group is selected as the representative chemical in the group with the best available dose-response data for all exposure routes under consideration. When

adequate data are available, RPFs are determined by dose-response modeling of a common end point (pertinent to the common mechanism of action) to arrive at a POD (such as a BMD_{10}) and dividing the POD for each component by that of the index chemical (a unitless number). Concentrations of member components in food and environmental media are converted to index chemical equivalents by multiplying the concentrations by the appropriate RPF, and then equivalent concentrations are summed. The summed equivalent concentrations are used in exposure models to estimate total index chemical equivalent intakes for target populations for multiple pathways and exposure scenarios. The ratio between the POD for the index chemical ($POD_{index\ chemical}$) and the total index chemical equivalent intake (i.e., exposure) is termed the MOE, which is used as EPA OPP's indicator of risk:

$$\text{Margin of exposure (MOE)} = POD_{index\ chemical} \div \text{exposure to index chemical equivalents}$$

Uncertainties in the exposure assessment and toxicity database are considered in selecting a suitable target MOE to indicate concern for increased risk of the common effect and characterizing the risk for target populations and exposure scenarios. An illustration of the whole process can be found in the description of the EPA (2007b) cumulative risk assessment for N-methyl carbamates in Appendix C.8 of this document. Wilkinson et al. (2000) have argued that the POD/MOE approach is more transparent than the hazard index approach, because the application of data-derived uncertainty factors and default policy-driven uncertainty factors are separated in the POD/MOE approach, but masked within the RfDs or MRLs used in the hazard index approach.

3.3.6. Total Cancer Risk Approach

A response-addition approach has been recommended for the assessment of risk from mixtures of carcinogenic chemicals (De Rosa et al. 1993; EPA 1986, 2000; Mumtaz et al. 1994a; NRC 1989). The most conservative form of response addition, completely negative correlation of tolerances (i.e., individuals most sensitive to chemical A are least sensitive to chemical B and vice versa) was recommended by EPA (1986). Accordingly, the response or risk for the mixture is the sum of the risks for the components:

$$Risk = \sum_{i=1}^{n} Risk_i = \sum_{i=1}^{n} d_i B_i \qquad (5)$$

where $Risk_i$ is the risk, d_i is the dose, and B_i is a potency parameter (slope factor or unit risk) for the i^{th} carcinogen. The equation is appropriate when risks for the individual chemicals are <0.01 and the sum of the individual risks is <0.1 (EPA 1989a). This equation is equivalent to dose addition if the dose-response curves for the chemicals are within the linear (low-dose) range, and have no threshold (EPA 1986, 2000). EPA (2000) recommends the response-addition model for independent action (as in Equation 18 of Appendix A) for cancer risk, noting that when component risks are small, the formula collapses to the simple addition of component risks (Equation 5 above). Use of the IRIS values for slope factor or unit risk result in plausible upper bounds to the lifetime excess cancer risk of the components. Concern has been raised that summing upper bound risks may lead to unreasonably high estimates of the mixture risk, but an analysis by Kodell and Chen (1994) suggested that the error in the simple sum of the upper bound risks is small relative to other uncertainties, and Cogliano (1997) concluded that the sum of the upper bound risks provides useful information regarding the overall risk from mixtures of carcinogens.

3.3.7. Applications of PBPK and PBPK/PD Models to Chemical Mixture Assessments

PBPK models for single chemicals are biological models that incorporate pharmacokinetic information (i.e., about absorption, distribution, metabolism, and elimination; also known as toxicokinetic information) to estimate internal doses of a chemical in the body from externally applied doses or concentrations. PBPK/PD models also incorporate information about the response of target tissues or cells to the chemical (i.e., pharmacodynamic information) (Caldwell et al. 2012; Mumtaz et al. 2012; Tan et al. 2011). PBPK models for single chemicals have been used to better inform human health dose-response assessment extrapolations from high doses to low doses, across species (e.g., from rats to humans), and across durations and routes of exposure (Caldwell et al. 2012; EPA 2006a; Mumtaz et al. 2012). Examples of toxicity guidance values that were developed using PBPK models for single chemicals include the EPA IRIS RfCs or cancer slope factors for dichloromethane (IRIS 2011a), trichloroethylene (IRIS 2011b), and vinyl chloride (IRIS 2003b), and the ATSDR MRLs for dichloromethane (methylene chloride) (ATSDR 2000a), 1,4-dioxane (ATSDR 2012b), cadmium (ATSDR 2012a), and trichloroethylene (ATSDR 2014). Single-chemical PBPK modeling is part of a process, termed quantitative *in vitro* to *in vivo* extrapolation, that is currently being investigated for use in extrapolating *in vitro* toxicity results to *in vivo* exposure scenarios via reverse dosimetry (Meek and Lipscomb 2015; Shin et al. 2015; Thomas et al. 2013; Wetmore 2015; Wetmore et al. 2012; Yoon et al. 2015).

Models for mixtures of two or more components have been developed by linking PBPK and/or PBPK/PD models for the individual components at points of potential pharmacokinetic or pharmacodynamic interaction, most commonly at hepatic metabolic inhibition (Andersen and Dennison 2004; Krishnan et al. 2002; El-Masri et al. 2004; Mumtaz et al. 2012; Tan et al. 2011). Following optimization and validation of the potential mechanisms of interaction by comparing model predictions of an internal dose metric or toxic outcome with experimental data, the mixture/interaction models have been used to investigate the dose-dependency of the magnitude of interactions and external exposure levels at which interactions (i.e., deviations from additivity) may or may not exist (Barton et al. 1995; Dobrev et al. 2001, 2002; Haddad et al. 1999a, 1999b, 2000a, 2000b; El-Masri et al. 1996, 2004; Krishnan et al. 2002; Pelekis and Krishnan 1997; Tardif et al. 1997). For example, Tardif et al. (1997) found that rat PBPK models for toluene, m-xylene, and ethylbenzene linked with competitive metabolic inhibition in the liver provided plausible agreement with results from gas uptake studies. Simulations of human models (scaled from the rat models) and results from volunteer studies showed that alveolar air concentrations and urinary metabolite concentrations, at exposure levels below permissible occupational exposure levels, were not significantly different between exposure to the individual components and exposure to the mixture. The results indicated the lack of an antagonistic metabolic interaction at these low exposure levels (Tardif et al. 1997). Similarly, El-Masri et al. (2004) demonstrated an interaction threshold for oral exposure of rats to a binary mixture of two organophosphorus insecticides that inhibit acetylcholinesterase after bioactivation by CYP enzymatic transformation: chlorpyrifos and parathion. Rat PBPK/PD models for each insecticide were developed to estimate blood concentrations of their metabolites and estimate kinetics of percent inhibition of free plasma acetylcholinesterase. A mixture model was developed that included interactions at: (1) the CYP enzymatic bioactivation step and (2) acetylcholinesterase binding sites. Model simulations with oral exposure to various dose levels of each insecticide alone and 1:1 mixtures indicated that the mixture model predicted responses that were increasingly smaller than the responses predicted by response additivity from the individual models at doses in the range of 1–10 mg/kg, thereby indicating antagonism (less-than-additive action) that increased with dose. No difference between the two methods became apparent at 0.08 mg/kg, the apparent interaction threshold for this binary mixture (El-Masri et al. 2004). Rat PBPK models for more complex mixtures of up to five volatile organic components (benzene, toluene, ethylbenzene, and xylenes [BTEX] and dichloromethane) have been developed that consist of PBPK models of the individual components linked by competitive metabolism in the liver (Haddad et al. 1999a, 1999b, 2000a, 2000b; Krishnan et al. 2002). The interaction-based mixture models adequately simulated measured internal dose metrics for each component following short-term inhalation exposure to various combinations and concentrations of the components. Simulations with a dichloromethane/BTEX model for humans (scaled from the rat model) indicated that competitive

metabolic inhibition would: (1) decrease hazard indices for anoxia (i.e., carboxyhemoglobin formation) from dichloromethane depending on mixing ratios with, and concentrations of, other components; (2) increase blood concentration x time profiles for each component at higher concentrations compared with dose additivity expectations with implications for prolonging acute central nervous system effects from all components; and (3) likely, at high concentrations, increase cancer risk from dichloromethane by shifting metabolism to a putatively cancer-related pathway (GSH conjugation vs. CYP) and decrease cancer risk from benzene by inhibiting formation of putatively carcinogenic reactive metabolites by CYP enzymes (Haddad et al. 2001). A noted limitation of applying this model to subchronic or chronic exposure scenarios is that it was developed with acute duration kinetics data and other points of metabolic interaction (e.g., enzyme induction) could arise with repeated exposure to the mixture (Krishnan et al. 2002).

An approach to dealing with very complex mixtures is to model fractions of the mixture as single components or lumps. This approach has been used to predict whether the metabolism of benzene to genotoxic metabolites is affected by the other components of gasoline in the mouse (Bond et al. 1998). A similar approach was proposed and partially developed for studying the acute toxicology of JP-5, a Navy jet fuel that contains a complex mixture of petroleum hydrocarbons in the C9–C18 range (Verhaar et al. 1997; Yang et al. 1998). The lumping concept for very complex mixtures has been applied to develop rat PBPK models for gasoline (Dennison et al. 2003, 2004). Results from gas-uptake studies of rats exposed for 6 hours to whole gasoline vapors or fractions of whole gasoline vapors were used to develop PBPK models that linked individual PBPK models for five individual components (n-hexane, benzene, toluene, ethylbenzene, and o-xylene) and a lumped component of the remaining evaporative components (principally hydrocarbons representing about 90% by weight of the complex mixture) by competitive metabolism in the liver. Stepwise optimization was used to estimate model parameters by comparison of time profiles of GC-measured chamber concentrations of the five individual components and the lumped component with model predictions. The internal dose metric of interest in these models was blood concentration of each individual component. Simulations with the developed rat models indicated that competitive metabolic inhibition for most of the components occurred at ≥300 ppm for whole gasoline vapors and ≥200 ppm for fractions of whole gasoline vapor that were expected to be more relevant to environmental exposure scenarios experienced by humans than whole gasoline vapors (Dennison et al. 2003, 2004).

To date, PBPK/PD models developed for mixtures have not been routinely applied in the development of risk-based guidance values for mixtures or cumulative risk assessments for specific mixtures. However,

ATSDR (2004b) used simulations from the BTEX PBPK model (Haddad et al. 1999a, 1999b, 2000, 2001; Krishnan et al. 2002) in support of recommendations for conducting exposure-based assessments of neurological, hematological, and cancer hazards from BTEX mixtures. Recommendations included a component-based hazard index approach that assumes dose additivity and uses ATSDR MRLs based on neurological impairment for neurological hazards, based on the implications from the PBPK model that joint neurotoxic action is dose additive at concentrations below about 20 ppm for each component. It was also recommended that the inhalation cancer slope factor for benzene be used for assessing cancer risk from BTEX exposures. ATSDR noted that the BTEX PBPK model simulations indicated that as exposure concentrations increase beyond 20 ppm for each component, the potential for neurotoxicity may increase and the potential for hematotoxicity/carcinogenicity may decrease beyond dose-additivity expectations due to competitive metabolic interactions among mixture components (ATSDR 2004b).

3.3.8. Approaches to Assessing Health Risks from Combined Exposure to Multiple Chemicals and Nonchemical Stressors

U.S. governmental agencies have responded to calls for developing guidance and guidelines for cumulative risk assessment for multiple chemical and nonchemical stressors (see Sexton [2012] for a historical review). Nonchemical stressors include biological (e.g., infectious microorganisms), physical (e.g., noise, vibrations), and psychosocial stressors (e.g., low socioeconomic status, dilapidated housing, residential crowding, and lack of access to health care). EPA (2003) prepared a *Framework for Cumulative Risk Assessment* that described a simple, flexible structure for conducting cumulative risk assessments, meaning "an analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors." The framework described three main phases to cumulative risk assessments (i.e., planning, scoping, and problem formulation; analysis; and risk characterization), but did not describe specific protocols or guidance for any of these phases. The EPA framework document, however, acknowledged a shift in its risk assessment processes to: (1) focus on identifying at-risk communities in contrast to the traditional focus of quantitatively estimating hypothetical individual risks for maximally exposed individuals from point sources or other types of environmental exposures to single or multiple chemicals; (2) use of qualitative or semi-quantitative data, such as broad exposure or toxicity indicators, in cases where the complexity of exposure and data deficiencies may hinder quantitative approaches; and (3) incorporate nonchemical stressors. The EPA viewed the framework as a first step in the long-term development of such guidance, and noted that incorporating "nonconventional stressors or risk factors (e.g., lifestyle, access to health care)" would need continued research. A report from a committee of the NRC (2009) reinforced the need for additional research to aid the development of cumulative risk assessment methods for multiple chemical and

nonchemical stressors, noting that EPA had not included, at that time, nonchemical stressors in quantitative or qualitative cumulative risk assessments.

The EPA (2003) discussion of incorporating nonchemical stressors into the analysis phase of cumulative risk assessments focused on a four-component concept of vulnerability of individuals or subgroups of the general population: (1) susceptibility or sensitivity related to biological differences associated with life-stage, genetics, or disease state; (2) differential exposure (i.e., disproportionate exposure relative to other groups or individuals) extending to historical exposure; (3) differential preparedness to withstand insult from a stressor; and (4) differential ability to recover from insults from a stressor (i.e., resiliency). Lack of access to health care, income differences, unemployment, or lack of insurance were given as examples of social factors that may influence a community's ability to prepare or recover from an insult from a stressor. Within this concept of vulnerability, Lewis et al. (2011) prepared a list of potential indicators of individual or community vulnerability compiled from several sources (Cal/EPA 2010; deFur et al. 2007; Morello-Frosch and Shenassa 2006; O'Neill et al. 2003). The list included indicators of biological susceptibility and sensitivity, such as inherited diseases, genetic polymorphisms, age, developmental or physiological stage (e.g., pregnancy), race/ethnicity/culture, mental health-related coping skills, and low intelligence or birth weight. Indicators of differential exposure related to either individual or community vulnerability included old/substandard housing, substandard sanitation, increased air pollutant exposure, and proximity to industrial release sites or hazardous waste sites. Indicators of either differential individual or community preparedness and recovery included low socioeconomic status, family instability, inadequate nutrition or food supply, limited health care access or insurance, high incidence of obesity, smoking or drug addiction, crime and violence, and lack of general community resources. Similarly, ATSDR (2014) developed a social vulnerability index approach that enables public health officials and emergency planners to identify and map communities that are socially vulnerable http://grasp.cdc.gov/grasp_intranet/grasp_svi.aspx Several efforts to develop tools to incorporate nonchemical stressors such as those associated with differential exposure and differential preparedness and ability to recover have been reported, but the tools are qualitative in nature and their usefulness is limited to ranking or prioritizing communities for further cumulative risk assessment investigations (Alexeeff et al. 2012; NJDEP 2009; Su et al. 2009). For example, The California Environmental Protection Agency is developing a screening tool for assessing differential cumulative impacts in different geographical regions incorporating chemical pollution data and community public health characteristics (Alexeeff et al. 2012). In a pilot analysis and application of the method to 30 ZIP mail code regions in California, ranking scores for exposure indicators (range of 1–10 based on PM2.5 and ozone air concentrations, EPA Toxics Release Inventory data, traffic volumes, and pesticide use), public health

effects (range of 1–5, based on data for birth weight, heart disease and cancer mortality, and asthma hospitalization), and environmental effects (range of 1–5, based on numbers of hazardous waste and clean-up sites and spills and leaks from underground fuel tanks) were added together and then multiplied by the sum of scores for sensitive populations (score of 1–3, based on census data for percent <5 and >65 years of age) and socioeconomic factors (score of 1–3, based on percent with less than a high school education, median household income, and percent below 2 times the national poverty level). Cumulative impact scores could range between 6 and 120. Alexeeff et al. (2012) emphasized that the method does not quantitatively estimate human health risks, but is a screening-level ranking tool. In another approach, Su et al. (2009) used air contaminant concentration data for three pollutants (NO_2, PM2.5, diesel particulate matter) to estimate environmental hazards and percentage of residents who are non-white or percentages of residents with incomes lower than 2 times the national poverty level to measure socioeconomic characteristics of census-based tracts (i.e., regions) within Los Angeles county and related the cumulative environmental hazards (combined either with a population-weighted multiplicative model or an additive model) to either of the socioeconomic characteristics with a ranking index (termed a cumulative hazard inequality index) to explore demographic inequalities in environmental hazard. The calculated indices reinforced the concept that demographic inequalities existed using either socioeconomic characteristic. Lewis et al. (2011) noted that these ranking approaches do not quantitatively attribute relative contributions of chemical and nonchemical stressors to health risks, and that additional research is needed to develop such quantitative approaches.

Physical stressors known to affect similar target organs as chemicals are likely to be incorporated into quantitative health impact or risk assessments with multiple chemical stressors sooner than psychosocial stressors, because methods to measure the intensity of exposure to physical stressors are available and characterization of dose-response relationships is thus more straightforward (Rider et al. 2014). Physical stressors with evidence that they can produce toxicity or modify the toxicity of chemicals include sunlight, noise, radiation, and temperature (Rider et al. 2014), but quantitative cumulative risk assessments including these stressors with chemical stressors that may affect similar toxicity targets are rare.

A recent case study shows how these assessments may proceed. Evans et al. (2014) conducted a screening-level cumulative risk assessment for potential hearing impairment from joint exposure to traffic-related noise and airborne concentrations of three volatile organic compounds (VOCs) (toluene, ethylbenzene, and mixed isomer xylenes) in San Francisco County, California. A component-based hazard index approach was used based on a dose-additivity assumption for these four components

determined to cause hearing impairment in other epidemiology and/or toxicology studies. Acceptable or "safe" levels for chronic exposure to each of the components were used to calculate HQs for each component: 70 decibels (dB) for noise as determined by WHO (1999) and EPA IRIS RfCs of 5 mg/m^3 for toluene (IRIS 2007), 1 mg/m^3 for ethylbenzene (IRIS 1998b), and 0.1 mg/m^3 for xylenes (IRIS 2003c). A noise map for San Francisco County was developed using a model of traffic-induced noise levels developed by the U.S. Federal Highway Administration. Geographical block groups within the county as determined by the 2000 U.S. census were the geographical units for the assessment. To estimate noise levels within each block group, the modeled noise level of streets within each block were averaged and placed into one of four noise categories: 45–60, 61–65, 66–70, and 71–75 dB. Because appropriate block-level data were not available for airborne concentrations of the VOCs, these were estimated (extrapolated) by quantile regression modeling of sociodemographic data (race, gender, education, and smoking status: these types of data were also available for the San Francisco block groups) and personal air VOC concentration data from 648 individuals with both types of data in the 1999–2000 U.S. National Health and Nutrition Examination Survey (NHANES). Hazard indices for each block were calculated by adding the block-level HQs for noise, toluene, ethylbenzene, and mixed xylenes. County-averaged hazard indices ranged from 0.8 for the tenth percentile of combined VOC exposure and the low noise category (45–60 dB) to 1.7 for the 90^{th} percentile of combined VOC exposure and the high noise category (71–75 dB).

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APPENDIX A. BACKGROUND INFORMATION ON THE ASSESSMENT OF ADDITIVITY AND INTERACTIONS

A.1. INTRODUCTION

The approaches to assessing the joint action of components of a mixture are based in large measure on the conceptual groundwork laid by Bliss (1939) and Finney (1971), and are mathematical rather than biological in nature. The approaches commonly known as dose addition and response addition, discussed in the following sections, are non-interactive forms of joint action that assume the chemicals in the mixture do not affect the toxicity of one another (i.e., that they act independently). These assumptions are the bases for methods of risk and health assessment discussed in this Framework Manual. In addition, the assessment of interactions depends on being able to define what constitutes non-interaction.

The available studies of toxicological interactions often pose a problem for the environmental scientists because the results may be ambiguous, often due to poor study design, or the results of several studies on the same mixture may appear to be conflicting, or the relevance of the study or studies to the exposure scenario of interest is uncertain. Approaches for dealing with these uncertainties are introduced in this appendix and further discussed in Appendices B and C.

A.2. MODELS FOR JOINT ACTION

A.2.1. Dose Addition

As introduced in this Framework, dose addition, also known as concentration addition, simple similar action, and similar joint action, assumes that the components of a mixture behave as concentrations or dilutions of one another, differing only in their potencies (Bliss 1939; Finney 1971). The dose-response curves are parallel (i.e., the regression lines of probits on log doses are parallel), and tolerance (or susceptibility) to the components is completely positively correlated (the organisms most susceptible to chemical A also will be most susceptible to chemical B). The response to the mixture can be predicted by summing the doses of the components after adjusting for the differences in potencies. Dose addition is considered most appropriate for mixtures with components that affect the same end point by the same mechanism of action (EPA 1986, 1988, 2000). It has been suggested that the requirement for parallel dose-response curves and complete correlation of tolerances may be too stringent (e.g., Plackett and Hewlett 1952; Svendsgaard and Hertzberg 1994), and that in the low-dose region in which the response is linear, dose additivity may hold for independently-acting chemicals as well (Svendsgaard and Hertzberg

1994). Dose addition is the underlying assumption of the hazard index method, the TEF approach for CDDs, and RPF approaches for carcinogenic effects from PAHs and neurological effects from groups of insecticides with common mechanisms (Section 3.3.5).

The regression lines for two chemicals (1 and 2) that act in a dose-additive manner can be represented as:

$$Y_1 = \beta \log x + \alpha_1 \quad (1)$$

$$Y_2 = \beta \log x + \alpha_2 \quad (2)$$

where x is dose or concentration, Y_i is the probit response for the i^{th} chemical, β is the slope (by definition the same for both chemicals), and α_i is the intercept on the exposure axis (the value of Y when x is zero) for the i^{th} chemical. The potency ρ of chemical 2 relative to chemical 1 is:

$$\log \rho = \frac{(\alpha_2 - \alpha_1)}{\beta} \quad (3)$$

Using Equation 3 to convert the dose of the second chemical into an equivalent amount of the first, Equation 2 can be rewritten as:

$$Y_2 = \beta \log(\rho \bullet x) + \alpha_1 \quad (4)$$

Thus, for a mixture of chemicals 1 and 2 in which the exposures are x_1 and x_2, the response is dose additive if it equals that produced by a dose ($x_1 + \rho x_2$) of the first chemical alone, as expressed by the following equation:

$$Y = \alpha_1 + \beta \log(x_1 + \rho \bullet x_2) \quad (5)$$

Alternatively, if the mixture is regarded as a total dose x, in which the proportions of the two chemicals are π_1 and π_2, Equation 5 can be written as:

$$Y = \alpha_1 + \beta \log(\pi_1 + \rho \pi_2) + \beta \log x \quad (6)$$

Equations 5 and 6 can be generalized for a greater number of components.

Relationships that may be useful in analyzing interactions data (Finney 1971) can be derived from Equation 6. If for a mixture of defined proportions of chemical 1 and 2, some uniform measure of toxicity (risk-specific dose or equally effective dose, e.g., ED_{50}) is known for the two chemicals and designated by ζ_1 and ζ_2, respectively, then:

$$\zeta_2 = \frac{\zeta_1}{\rho} \tag{7}$$

The toxicity ζ_m of any mixture of chemicals 1 and 2 can be predicted as follows under the assumption of dose addition:

$$\zeta_m = \frac{\zeta_1}{(\pi_1 + \rho\pi_2)} \tag{8}$$

Equation 8 can also be written in the following form:

$$\frac{1}{\zeta_m} = \left(\frac{1}{\zeta_1}\right)\pi_1 + \left(\frac{\rho}{\zeta_1}\right)\pi_2 \tag{9}$$

Based on equation 7, $1/\zeta_2$ can be substituted for ρ/ζ_1 in Equation 9 to give:

$$\frac{1}{\zeta_m} = \frac{\pi_1}{\zeta_1} + \frac{\pi_2}{\zeta_2} \tag{10}$$

This form of the equation can be used to predict the ED_{50} (or other uniform measure of toxicity) of a mixture from the proportions and ED_{50}s of the components.

A.2.2. Applications of Dose Addition to Health and Risk Assessment

The TEQ approach and hazard index approach are based on the assumption of dose addition. The response to the mixture is considered dose additive if it equals that produced by a dose of the first chemical alone. The mixture dose (X), expressed as an equivalent dose of the first chemical alone, is:

$$X = \rho_1 x_1 + \rho_2 x_2 + \rho_3 x_3 + \cdots + \rho_n x_n \quad (11)$$

where ρ_i is the potency of the i^{th} component relative to the first chemical and x_i is the concentration or dose of the i^{th} component. Note that $\rho_1 = 1$, the potency of chemical 1 relative to itself.

In the TEQ approach for CDDs and related compounds, the first or index chemical is 2,3,7,8-TCDD, which is assigned a TEF of unity, representing its potency relative to itself. TEFs for the other congeners are based on their potency relative to 2,3,7,8-TCDD. The concentrations or doses of all active congeners are multiplied by their TEF values and summed to give the TEQs for the mixture, which is the concentration of the mixture expressed as an equivalent concentration of the index chemical, 2,3,7,8-TCDD:

$$TEQs = TEF_1 C_1 + TEF_2 C_2 + TEF_3 C_3 + \cdots + TEF_n C_n = \sum_{i=1}^{n} TEF_i C_i \quad (12)$$

where TEF_i is the potency of the i^{th} component relative to 2,3,7,8-TCDD and C_i is the concentration of the i^{th} component (ATSDR 1998b; EPA 2010b; Van den Berg et al. 2006). Equation 12 is equivalent to Equation 5 of the Framework manual.

The relative potency method for PAHs (ATSDR 1995b; EPA 1993) is a similar application of dose addition. Additional information and references are provided in Section 3.3.5 of the Framework manual.

The hazard index approach uses 1/DL (where DL is a defined level of exposure such as an MRL or RfD) as an indicator of potency (because the larger the DL, the less the potency) for the components of a mixture. If E is the total mixture dose or exposure expressed as the equivalent dose of chemical 1, where chemical 1 can be any component of the mixture, then, under dose addition:

$$E = \frac{DL_1}{DL_1} E_1 + \frac{DL_1}{DL_2} E_2 + \frac{DL_1}{DL_3} E_3 + \cdots + \frac{DL_1}{DL_n} E_n \quad (13)$$

where DL_i is the defined level for the i^{th} component, and E_i is the exposure to the i^{th} component, in the same units.

Factoring out DL_1 from the numerators, Equation 13 becomes:

$$E = DL_1\left(\frac{E_1}{DL_1} + \frac{E_2}{DL_2} + \frac{E_3}{DL_3} + \cdots + \frac{E_n}{DL_n}\right) \quad (14)$$

Dividing both sides of Equation 14 by DL_1 gives the expression for the hazard index (*HI*):

$$\frac{E}{DL_1} = HI = \frac{E_1}{DL_1} + \frac{E_2}{DL_2} + \frac{E_3}{DL_3} + \cdots + \frac{E_n}{DL_n} \quad (15)$$

The hazard index approach is discussed in Section 3.3.2 of the Framework manual.

Limitations of the hazard index approach include the requirement imposed by the dose addition model that the MOA of the chemicals be similar, and the weakness of the assumption that the defined levels (MRLs or RfDs) represent isoeffective doses. Potential improvements to the approach include the use of toxicity thresholds or BMD or effective dose levels (e.g., BMD_{10} or ED_{10} values), rather than MRLs or other defined levels. The EPA OPP's (EPA 2002b) approach for cumulative risk assessment of common effects from groups of pesticides sharing a common mechanism uses an RPF approach linked with a POD (e.g., BMD_{10})/MOE approach to characterizing the risk. See Sections 3.3.5 and 4.8 for more details on how this approach incorporates uncertainty factors into these assessments.

A.2.3. Response Addition

Response addition, as introduced in Section 3.3.1, also known as simple independent action and independent joint action (Bliss 1939), assumes that the chemicals act independently and by different MOAs. Because the MOAs are different, tolerance (or susceptibility) to the components is not necessarily positively correlated under response addition. The response to the mixture (expressed as the percent in a population responding) can be predicted from the responses to the individual components and the correlation of tolerance distributions (also termed susceptibility distributions) among components of the mixture (the proportion of members of a population responding as the exposure level of the component increases). Response addition is the underlying assumption of an approach to cancer risk assessment for mixtures at Superfund sites, EPA's and ATSDR's approach to noncancer risk assessment when exposure levels for components are near the individual NOAELs from well-designed toxicology

studies, and ACGIH's approach to assessing the hazards of occupational exposure to agents that act independently.

The form of response addition for populations will be different depending on the correlation of susceptibility to the components of the mixture. If the individuals most sensitive to chemical 1 are also most sensitive to chemical 2, susceptibilities to chemicals 1 and 2 are completely and positively correlated. The correlation coefficient r is equal to one. The expected response P to the mixture of chemicals 1 and 2 at doses that individually produce responses P_1 and P_2 is equivalent to that for the chemical with the highest response. Thus:

$$\begin{aligned} P &= P_1 \quad if\ r=1 \quad P_1 > P_2 \\ P &= P_2 \quad if\ r=1 \quad P_2 > P_1 \end{aligned} \tag{16}$$

In other words, if the dose of chemical 1 would be expected to cause a response in 8% of individuals and chemical 2 would be expected to cause a response in 17% of individuals, the expected response to the mixture of these two chemicals at these doses is 17% when susceptibilities are completely positively correlated.

If the individuals most sensitive to chemical 1 are least sensitive to chemical 2 and vice versa, susceptibilities to chemicals 1 and 2 are completely and negatively correlated. Under this circumstance, the predicted response of the population to the mixture would be simply additive (8 + 17 = 25%) as long as the total of the responses to chemicals 1 and 2 was less than unity.

$$P = P_1 + P_2 \quad if\ r=-1 \qquad (P_1 + P_2) \leq 1 \tag{17}$$

Intermediate to these two extremes is the circumstance when the susceptibility to the two chemicals are statistically independent; the order of individuals showing toxic effects from chemical 1 has no apparent relationship with the ordering of individuals showing toxic effects from chemical 2 (r=0). In this case, some of the organisms that would not respond to chemical 1 would respond to chemical 2, so that the total response rate for the mixture is:

$$\begin{aligned} P &= P_1 + P_2(1 - P_1) \\ &= P_1 + P_2 - P_1P_2 \end{aligned} \tag{18}$$

Using the same response rates as in the previous examples, the response to the mixture would be estimated as $100(0.08 + 0.17 - (0.08 \cdot 0.17)) = 23.6\%$. The general form of the equation for multiple component mixtures is: $P_{mixture} = 1- (1-P_1) * (1-P_2) * (1-P_3) \ldots..$

The above equations can be generalized for a greater number of components. EPA (2000) commented that response addition formulas for populations, as illustrated in the examples above, have received limited use in risk assessment because detailed data for tolerance distributions are often not available and the concepts of tolerance correlation only works well if there are two chemicals in a mixture. Nevertheless, several applications of response addition assumptions are described in the next section.

A.2.4. Applications of Response Addition to Health or Risk Assessment

An approach similar to response addition assuming completely positive correlation of tolerances (Equation 16 of this appendix for a two-component mixture: $P_{mixture} = P_1$, if $r = 1$ and $P_1 > P_2$; $P_{mixture} = P_2$, if $r = 1$ and $P_2 > P_1$) has been applied by ACGIH to the assessment of mixtures whose components are expected to cause effects that are independent from each other, such as purely local effects on different organ systems. The threshold limit for the mixture is considered to be exceeded only if the HQ for at least one of the components exceeds unity (Section C.1).

The calculation of total cancer risk based on response addition with completely negative correlation of tolerances has been recommended as an approach for adding cancer risks for mixtures of a few chemicals (EPA 2000; see Chapter 2). The responses (risks) for the individual components of the mixture are summed to estimate the response to the mixture as in equation 17 of this appendix. EPA (2000) recommended that the full general equation for independently acting carcinogens [i.e., $P_{mixture} = 1- (1-P_1) * (1-P_2) * (1-P_3) \ldots..$] be applied to mixtures with more than a few carcinogens.

For low exposure levels (i.e., levels near individual component NOAELs from well-designed toxicology studies), toxicologically dissimilar chemicals are assumed to be independent, and response addition is assumed to be useful for noncancer risk assessment by EPA (2000) and this ATSDR framework (see Chapter 2). As such, when exposure to each component of a mixture is below the RfD, RfC, or MRL (estimated risk from each component = 0), the risk of adverse outcome from the mixture is usually assumed to be negligible. EPA (2000) noted that in these cases, "0 is used to denote a risk that is either subthreshold (a true zero risk) or small enough to be general considered virtually safe." EPA (2000) further noted that when the number of components in a mixture is large, and all component exposures are

close to, but below, respective guidance values (RfD, RfC, or MRL), “the toxicity data should be carefully examined to ensure that all effects and MOAs are being considered when deciding functional independence.” With poor quality data supporting the guidance value or the exposure assessment, “the conclusion of negligible risk is similarly uncertain (EPA 2000).”

A.3. INTERACTIONS

A.3.1. Introduction to Interaction Models

The assessment of interactions involves assumptions regarding what constitutes an additive or non-interactive response. Thus, the assumed form of additivity often drives experimental design and the assessment of joint action. Knowledge of the MOA of the individual components of the mixture is often used in selecting a plausible additivity model.

If interactions appear to exist, as determined from deviations from the assumed form of additivity, mathematic models for quantifying the interactions may be used. Finney (1942, 1971) proposed the following interaction model, which is a modification of Equation 5 for dose addition:

$$Y = \alpha_1 + \beta \log(x_1 + \rho \bullet x_2) + \kappa(\rho \bullet x_1 \bullet x_2)^{0.5} \quad (19)$$

where κ is the interaction coefficient. Positive values of κ indicate synergism, negative values indicate antagonism, and a value of zero indicates dose addition.

A.3.2. Early Experimental Studies Examining Dose Additivity

Experimental studies of toxicological interactions, particularly those designed primarily to investigate the mechanism of action of the chemical of interest, may not reflect the models discussed above. From the material already presented in this appendix, it follows that, in general, an understanding of the joint action of the components of a mixture depends upon an understanding of the dose-response relationships for the individual components. There are exceptions to this generalization. An example is the case where one component is known to be inactive with regard to the effect of concern. In this case, only the dose-response curves for the active component with and without the addition of the inactive component may be necessary.

Other interaction studies do use dose addition or response addition models in the evaluation of additivity versus interactions. For example, Smyth et al. (1969) used Equation 10 to predict the toxicity (LD_{50}) of the 350 possible binary mixtures of 27 industrial chemicals administered in equivolume combinations. (One pair of chemicals proved impossible because it reacted vigorously upon mixing before administration.) The ratio between the predicted (*P*) and observed (*O*) values, calculated for each pair, ranged from 0.23 to 5.09, indicating that the magnitude of deviation from dose additivity was approximately a factor of ≤5. This is not a remarkable deviation from additivity and thus suggests that dose additivity is a reasonable default model for joint action. The upper end of the range of the deviation from additivity of 5 also has been used as the basis for a default magnitude of interaction factor in the modified WOE method (EPA 2000) described in Appendix B. Smyth et al. (1970) retested 53 chemical pairs from this set in equitoxic combinations. Because the distribution of ratios for the first (equivolume) study was skewed, the investigators normalized the ratios in that study and in the equitoxic study using the following adjustment:

where $P/O>1$; adjusted ratio = $(P/O) - 1$
where $P/O<1$; adjusted ratio = $1 - (O/P)$

With the adjusted ratios, a positive value indicates greater-than-additive joint action, a negative value indicates less-than-additive joint action, and a value of zero indicates additivity.

The equivolume and equitoxic experiments used different proportions of the chemicals for each pair. The difference in proportions should not affect the ability of equation 10 to predict the LD_{50} for the mixture. A comparison of the adjusted ratios in the equivolume and equitoxic experiments on the same pairs of chemicals showed that the correlation between the two sets of ratios was good. These results further support dose addition as a reasonable default model for joint action.

A.3.3. Evaluating Interaction Studies

To assess potential additivity and interactions that may occur among chemicals in a mixture and the effect that interactions will have on the inherent toxicity of the individual components of the mixture requires a thorough evaluation of the available studies on joint toxic action for the mixture and/or components of the mixture. The studies should be assessed based on the quality of the study and the applicability of the study design to predicting interactions or additivity.

ATSDR has adopted the NRC (1984) Guidelines for Assessing the Quality of Individual Studies, which appear in *Toxicity Testing: Strategies to Determine Needs and Priorities.* The NRC considers a report of scientific findings adequate for use in health hazard assessment if the report meets the following basic criteria:

1. All elements of exposure are clearly described.
2. Results in test subjects are predictive of human response, and test subjects are sensitive to the effects of the substance.
3. Controls are comparable with test subjects in all respects except the treatment variable.
4. End points answer the specific questions addressed in the study.
5. Observed effects are sufficient in number or degree to establish a dose-response relationship that can be used in estimating the hazard to the target species.
6. Both the design and the interpretation of the study allow for appropriate statistical analysis of the data.

Criteria for good studies further developed with the use of systematic reviews (Lynch et al. 2016; Rooney et al. 2014).

ATSDR recommends that good quality studies designed to assess the possible mode by which two or more chemicals affect a biological outcome should include:

1. Characterization of the effects of the individual components (and their dose-response relationships) on the outcome.
2. Generation of a hypothesis regarding the mode of joint action (e.g., dose addition or response addition).
3. Prediction of responses to mixtures of the components based on the postulated mode of joint action.
4. Observations of the response to mixtures of the components.
5. Statistical comparison of the predicted responses with the observed responses to the mixture.

These criteria are reflective of, and supplemented by, the criteria articulated by Borgert et al. (2001) for evaluating toxicological interaction studies:

1. Dose-response curves for the mixture components should be adequately characterized.

2. An appropriate “no-interaction” hypothesis should be explicitly stated and used as the basis for assessing synergy and antagonism.

3. Combination of mixture components should be assessed across a sufficient range (of exposure levels and mixing ratios) to support the goal of the study.

4. Formal statistical tests should be used to distinguish whether the response produced by a dose combination is different (larger or smaller) from that predicted by the ‘no-interactions” hypothesis (dose addition or response addition).

5. Interactions should be assessed at relevant levels of biological organizations.

An illustration of an adequate type of study involves two chemicals (A and B) that both individually affect a biological outcome. Dose-response data for each chemical alone indicate that linear dose-response models are adequate to describe dose-response relationships, and that A is 3 times more potent than B. Based on postulated joint additive action in which the null hypothesis is that the two chemicals behave as if they were concentrations or dilutions of one another (dose addition), a mixture of 1 dose unit of A plus 3 dose units B would be predicted to produce a response equivalent to that produced by:

- 2 dose units of A alone,
- 6 dose units of B alone, or
- a mixture of 0.33 dose unit of A and 5 dose units of B.

If observed responses to the mixture are greater than predicted responses, evidence is provided of a greater-than-dose-additive joint action. Conversely, if observed responses are less than predicted responses, there is evidence of a less-than-dose-additive joint action. If the dose-response relationships for the components and the mixture are not linear (e.g., show a sigmoidal shape), these specific predictions do not apply. With adequate characterization of the individual sigmoidal dose-response relationships, however, sufficient predictions of the combined effect by either dose addition or response addition can be calculated, and statistical tests comparing observed and predicted responses can be applied to assess deviations from either of these “no-interactions” hypotheses.

Unfortunately, the early toxicological literature on possible interactions among chemicals contains only limited numbers of studies that have all of the features of an optimal joint toxic action study. A standard design that was often followed (2x2 factorial design) involves a zero dose group (control), and chemicals A and B tested alone at doses of A1 and B1 and in combination at a dose of A1+B1. This type of design does not provide a full characterization of joint action, and the statistical analysis provided in such studies

often provides only information as to which treatment results are significantly different from other treatment results, rather than an indication of whether the results are indicative of a departure from dose addition or response addition (i.e., an interaction).

More complete discussion of statistical methods (and study design characteristics) to compare predicted and observed responses to mixtures are discussed by Berenbaum (1981), Bosgra et al. (2009), Calabrese (1991), Gennings et al. (2004, 2005), Hertzberg et al. (2013), Lutz et al. (2002), Scholze et al. (2014), and Svendsgaard and Hertzberg (1994).

Interaction studies also should be evaluated as to whether other components of the experimental design are relevant for assessing potential health outcomes of populations living near hazardous waste sites, including exposure route, duration of exposure, sequence of chemical administration, vehicle, dose and mixing ratio, and end points. Inhalation, oral, and dermal exposure are the most likely routes of exposure for human populations, and emphasis should be placed on interaction studies using these exposure routes. In the absence of data for a particular exposure route, data from other exposure routes may be used to predict interactions and health outcomes.

Use of data from another route would be based on the assumption that once a chemical has entered the body, there are no route-specific differences in toxicity or potency. However, this assumption may not be true if portal-of-entry effects or first-pass effects occur. First-pass effect refers to the metabolism that can take place in the portal-of-entry tissue, prior to entry into the systemic circulation, and can modulate the dose to remote or systemic target tissues in a route-dependent fashion. First-pass effect is usually considered with oral exposure because many chemicals are directly delivered from the gastrointestinal tract to the liver via the portal vein. The respiratory tract can also exhibit a first-pass effect after inhalation exposure. Although parenteral exposure is not an exposure route of concern, parenteral administration studies should be reviewed and evaluated if few or no studies using more relevant routes are available, because these data can provide valuable information on potential interactions and can provide mechanistic data. The relevance of parenteral studies to interactions involving oral exposure to the metals, however, needs careful consideration because parenteral administration bypasses homeostatic mechanisms and potential points of interaction related to absorption from the gastrointestinal tract.

Interactions among chemicals in a mixture can vary with duration of exposure. This is particularly true for chemicals that are toxic following chronic exposure but have low acute toxicity, or for chemicals whose biotransformation involves enzyme induction. When reviewing interaction data, the applicability

of the results to different exposure durations should be carefully considered. The toxicity/carcinogenicity and toxicokinetic databases for the chemicals of concern may provide useful information to support or refute extrapolation across exposure durations.

Interaction studies have utilized two patterns of administration: simultaneous and sequential. In the simultaneous administration study design, the mixture components are administered at the same time, or virtually the same time, using the same or different exposure routes. As this pattern of administration most closely resembles environmental exposure, greater emphasis should be placed on these data. Prior to 1991, many interaction studies employed a sequential pattern of administration, in which a chemical that alters metabolism or physiology in a known manner was administered before a single dose or exposure of the chemical of concern, in order to investigate the impact on the second chemical's toxicity (Hertzberg and Durkin 1994; Mumtaz and Durkin 1992). This study design provided data useful in elucidating the mechanism of action of the second chemical, but may not be as useful in understanding potential joint toxic action involving low-level, long-term simultaneous exposure.

Dose or exposure level and mixing ratio are also important factors to consider when evaluating interaction studies. In general, an understanding of the dose-response relationships for the individual components of the mixture is important for understanding potential interactions and health outcomes following exposure to the mixture. For example, if the dose tested is much lower than the threshold for the toxic end point of concern, then a potential interaction may not be detected by the study. On the other hand, if the dose used is too high, the dose may overwhelm the normal metabolic processes, resulting in different metabolites or an accumulation of a particular metabolite. Similarly, when examining potential interactions for a certain health effect, it is important to examine what other effects are occurring at the tested doses, and, in particular, whether the dose is so high that it is causing serious health effects in other organ systems, or death. Likewise, results from an interaction study evaluating a mixture with relative proportions of components different from the relative proportions in environmental mixtures may be of uncertain relevance to evaluating potential deviations from additivity in environmental mixtures.

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APPENDIX B. CHEMICAL INTERACTIONS WEIGHT-OF-EVIDENCE (WOE) METHODS

B.1 INTRODUCTION

The WOE methods for the assessment of chemical interactions described in this appendix were designed to facilitate the use of interactions data in the components-based assessment of noncancer health effects from exposure to chemical mixtures. As noted above, the hazard index method does not incorporate information on interactions among components of the mixture. A WOE method proposed by Mumtaz and Durkin (1992) was the first systematic attempt to address this need. The method implemented and expanded on the suggestion of the NRC (1989) that an uncertainty factor be used to account for interactions among components of a mixture. The value of the uncertainty factor can reflect the concern for interactions, and is modified using data regarding the WOE for interactions (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). As suggested by the NRC, the uncertainty factor is applied to the additivity-based hazard index to estimate an interactions-adjusted hazard index. Subsequent experience with the algorithm that is used to generate the interactions-adjusted hazard index has revealed, however, that it does not handle changes in the proportions of mixture components in a reasonable manner. The method remains useful in the qualitative prediction of whether a hazard may be greater or less than indicated by the hazard index (Sections B.1.2 and B.2.2).

A modification to the WOE method was developed by EPA (2000) in order to explicitly incorporate information on the magnitudes of the pairwise interactions into the risk assessment. This modified method addresses some of the limitations of the original method, but introduces a new set of limitations: (1) greater judgment may be required in the scoring of the WOE; and (2) information on the magnitude of interactions is rarely available.

An abbreviated description of the original method was presented in the main body of the Mixtures Guidance manual; some of the information will be repeated here for the sake of completeness and to facilitate comparison of the two methods. The following sections provide additional details of these methods.

B.2 ORIGINAL WOE METHOD

B.2.1. BINWOE Scores

The first step in applying the WOE method is to assess data relevant to joint action for each possible pair of chemicals in the mixture in order to make a qualitative BINWOE determination for interactions. The BINWOE determination is a classification that reflects the quality of the available information and categorizes the most plausible nature of the potential influence of one chemical on the toxicity of another chemical for a given exposure scenario (duration, route, and sequence). This determination includes evaluating information regarding the toxicity, pharmacokinetics, and mechanism of action of the individual chemicals; interactions data on each chemical pair; and interactions and mechanistic data on related chemicals. Although earlier publications of the WOE method did not discuss the need for target organ consideration in BINWOE determinations (Mumtaz and Durkin 1992), experience in application of the WOE method has indicated that the WOE evaluations should be target-organ specific (Mumtaz et al. 1998). Two BINWOE determinations are made for each pair: one for the effect of chemical A on the toxicity of chemical B, and the other for the effect of chemical B on the toxicity of chemical A (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). The criteria and scoring system for the BINWOE determinations are presented in Table B-1.

The classification of direction of interactions in Table B-1 has the following categories: additive, greater-than-additive, less-than-additive, and indeterminate. The additive category refers to results that are additive by a defined model of additivity (e.g., dose or response addition), and results that demonstrate no effect of one chemical on the toxicity of the other. The greater-than-additive category refers to synergism or potentiation. The less-than-additive category refers to antagonism, inhibition, or masking. Indeterminate refers to instances of ambiguous, conflicting, or no data.

The classification of the quality of the data in Table B-1 includes two main categories: mechanistic understanding and toxicological significance. The rating for mechanistic understanding reflects the quality of the available mechanistic data supporting a toxicological interaction and the extent to which this information indicates the direction of the interaction. Mechanistic information is information regarding the manner in which a chemical causes a given toxic effect or interaction, and may include chemical, biological, and physical processes at the molecular level and at higher levels of biological or physiological organization. The rating for toxicological significance reflects the quality of the available toxicological interactions data and the extent to which it indicates that the chemicals will interact in a manner that significantly impacts the health of the exposed population. Both the mechanistic and

toxicological categories allow for, and encourage, the use of structure-activity data in reaching conclusions. The modifiers in Table B-1 are used when the mechanistic and toxicological ratings do not account for the additional concerns for differences in duration, sequence, bioassay (*in vitro* versus *in vivo*), or route of exposure between the site-specific exposures and the mechanistic and toxicological data used for the BINWOE determinations (Mumtaz and Durkin 1992).

Table B-1. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions

	Classification	Factor
Direction of Interaction		**Direction**
=	Additive	0
>	Greater than additive	+1
<	Less than additive	−1
?	Indeterminate	0
Quality of the Data		**Weighting**
Mechanistic Understanding		
I.	Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.	1.0
II.	Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur have not been well characterized for the chemicals of concern but structure-activity relationships, either quantitative or informal, can be used to infer the likely mechanisms(s) and the direction of the interaction.	0.71
III.	Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.	0.32
Toxicological Significance		
A.	The toxicological significance of the interaction has been directly demonstrated.	1.0
B.	The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.	0.71
C.	The toxicological significance of the interaction is unclear.	0.32
Modifiers		
1.	Anticipated exposure duration and sequence.	1.0
2.	Different exposure duration or sequence.	0.79
a.	*In vivo* data	1.0
b.	*In vitro* data	0.79
i.	Anticipated route of exposure	1.0
ii.	Different route of exposure	0.79

Weighting factor = product of weighting scores: maximum = 1.0, minimum = 0.05

BINWOE = direction factor x weighting factor: ranges from −1 through 0 to +1

Sources: Mumtaz and Durkin 1992; Mumtaz et al. 1994a

The qualitative direction and alphanumeric data quality terms are shown in the left column of Table B-1. The corresponding direction factor and numerical data quality weighting factors are shown in the right column. The qualitative scores can be converted to a single numerical score by multiplying the direction factors (labeled Direction in the table) and the data quality weighting factors (labeled Weight in the table). Thus, an alphanumeric (qualitative) BINWOE classification of >II.B.2.a.i. corresponds to greater-than-additive interaction, mechanistic data on related chemicals, inferred toxicological significance, different duration or sequence, *in vivo* data, and anticipated route of exposure. The corresponding numerical BINWOE score is +1(0.71)(0.71)(0.79)(1)(1) = +0.40.

The data quality weighting factors were selected using the following reasoning: the optimum score for data quality is unity, and corresponds to the first level of scoring (categories I and A for the primary classifications of mechanistic or toxicological significance and 1, a, and I for the modifiers). For the primary classifications, the value of 0.71 was selected for the second level of scoring (categories II and B) so that if both factors were selected, the score would be about one-half of the optimum score ($0.71 \cdot 0.71 \approx 0.50$). Similarly, for the third level of scoring (categories III and C), the value of 0.32 was selected so that if both factors were selected, the score would be about one-tenth of the optimum score ($0.32 \cdot 0.32 \approx 0.1$). For the modifiers, a value of 0.79 was selected for the second level of scoring (2, b, and ii) so that all three factors combined would lower the score by a factor of about 0.5 ($0.79 \cdot 0.79 \cdot 0.79 \approx 0.5$). The numerical weighting values reflect judgment as to the relative importance of the data quality classifications in determining the WOE (Mumtaz and Durkin 1992).

The BINWOE determinations do not explicitly consider the relevance of dose to the anticipated exposure scenario. It is not uncommon to find that, for a well-studied binary mixture, the available information suggests that no interactions occur at low doses, but that an interaction, either greater-than-additive or less-than-additive, occurs at higher doses. The BINWOE for this situation would reflect the interaction observed at higher doses. Dose *is* taken into account in the calculation of interaction factors (Section B.2.2). Additional guidance for the determination of BINWOEs is provided in the ATSDR *Guidance for the Preparation of an Interaction Profile* (ATSDR 2001).

B.2.2. Qualitative WOE Method

A qualitative WOE approach, focusing on application of the BINWOE scores to hazardous waste site assessment, was suggested by Mumtaz and Durkin (1992). This approach is appropriate for a mixture where the scaled doses (HQs) for all of the components are similar, or toxicologically significant. The qualitative BINWOE scores for the components, if similar in direction, are the basis for a conclusion. For example, consider a mixture of four components, all present at toxicologically significant levels. The number of possible chemical pairs in a mixture of N components is $(N^2-N)/2$. Thus, this mixture of 4 components has 6 pairs of components and potentially 12 BINWOEs. Suppose nine of the BINWOEs are greater-than-additive (positive) with alphanumeric classifications indicating a relatively high degree of confidence, and the remaining three BINWOEs are additive (0), also with relatively high degrees of confidence. In this case, the WOE suggests that the mixture is likely to pose a greater hazard than that indicated by the hazard index.

A likely pattern of qualitative BINWOEs for a mixture is a mixed pattern (some greater-than-additive, some less-than-additive, and some additive BINWOEs). In this case, the qualitative WOE approach is extended to include conversion of the qualitative BINWOE scores to numerical scores, and summing the scores to give a combined score. If the combined BINWOE score is positive and significantly different from zero, then the WOE suggests that the mixture is likely to pose a greater hazard than indicated by the hazard index. Conversely, if the combined BINWOE score is negative and significantly different from zero, then the WOE suggests that the health hazard is unlikely to be greater than indicated by the hazard index. Professional judgment is used in the interpretation of the impact of the WOE on the hazard index.

Although the above WOE method was developed for assessing interactions for noncarcinogenic effects, the qualitative WOE method is equally applicable to assessing interactions for carcinogenic effects.

B.2.3. Interaction Factors

The quantitative application of the WOE method is described in this section, and continues through Section B.2.5. As mentioned previously, this quantitative application does not handle changes in the proportions of mixture components in a reasonable manner, and is no longer in use. The description is retained in this document because the method represents an interesting and original attempt to modify the hazard index for interactions.

In this quantitative application, the BINWOEs are used as interaction terms in the calculation of interaction factors, $IF_{i,j}$ and $IF_{j,i}$ (where $IF_{i,j}$ is the effect of j on the toxicity of i and $IF_{j,i}$ is the effect of i on the toxicity of j) as follows:

$$IF_{i,j} = \frac{HQ_i}{HI_{add}} \bullet BINWOE_{i,j} (HQ_i \cdot HQ_j)^{0.5} \quad (1)$$

$$IF_{j,i} = \frac{HQ_j}{HI_{add}} \bullet BINWOE_{j,i} (HQ_i \cdot HQ_j)^{0.5} \quad (2)$$

The two equations are identical except that Equation 1 calculates the interaction factor for the effect of j on the toxicity of i, and Equation 2 calculates the interaction factor for the effect of i on the toxicity of j.

The first set of terms in these equations weights the interaction factor by the contribution of the chemical whose toxicity is affected to the total toxicity of the mixture, expressed as the ratio of the HQ (HQ_i) of that chemical to the total additivity-based hazard index (HI_{add}) of the mixture (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). This approach is adapted from one developed by Durkin (1981) to account for asymmetrical interactions under the assumption of dose additivity. Asymmetrical interactions are those in which the magnitude of the interaction, and sometimes the direction of the interaction, vary with the proportions of the components in the mixture.

The BINWOE score is the interaction term that quantifies concern for interaction between a chemical pair. Estimation of the BINWOE score was discussed in the previous section.

The last set of terms in these two equations is the geometric mean of the HQs for the two chemicals. Finney (1942, 1971) proposed a similar term for modeling symmetrical interactions under the assumption of dose additivity. The use of the geometric mean lowers the value of the interaction factor as exposure to either of the two chemicals falls below the defined level (denominator of the HQ; e.g., MRL) for that chemical (i.e., as either HQ falls below unity). This property of the WOE approach is consistent with the general observation that as exposure levels and the probability of responses due to the individual components decrease, the toxicological significance of interactions in a mixture will decrease (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). In addition, the use of the geometric mean lowers the value of the interactions factor as the HQs of the two components deviate from each other. This is consistent with the assumption that the greatest departure from additivity (greatest interaction) will occur when both

components of a binary mixture are present in equitoxic amounts. This assumption also is expressed in Finney's model of a deviation from dose additivity (Finney 1942, 1971), presented in Appendix A.

B.2.4. WOE

The next step in this method is to sum the interaction factors to express the overall direction and WOE for the toxicological interactions of the site-specific mixture, WOE_S.

$$WOE_S = \sum_{i \neq j}\sum IF_{i,j} \quad (3)$$

The double summation sign indicates that each component of the mixture is evaluated for the effect that every other component could have on its toxicity. The overall process (substituting the full expression for the interaction factors into Equation 3) can be represented by Equation 4.

$$WOE_S = \sum_{i \neq j}\sum \frac{HQ_i}{HI_{add}} \cdot BINWOE_{i,j} \cdot (HQ_i \cdot HQ_j)^{0.5} \quad (4)$$

The WOE_S score has no absolute or clear interpretation. For example, a score of -0.16 could be a composite of interaction factors for antagonism (-0.223) and synergism (+0.060) or a composite of interaction factors all of which reflect very low confidence in antagonism (e.g., -0.01, -0.04, -0.05, -0.01, -0.02, -0.03). Therefore, Mumtaz and Durkin (1992) recommended that the WOE be normalized by dividing the WOE_S by the maximum possible score that the site-specific mixture would have generated if all of the interaction information had indicated a consistent direction of interaction and had been assigned weighting scores indicating the highest possible degree of confidence (BINWOE determinations of I.A.1.a.i. with corresponding BINWOE scores of 1.0). Because the BINWOE scores are 1, they essentially drop out of Equations 1 and 2 for the interactions factors, and therefore out of Equation 4. Accordingly, the maximum possible score, WOE_{MAX}, can be calculated by summing the simplified expressions for the interaction factors as follows:

$$WOE_{MAX} = \sum_{i \neq j}\sum \frac{HQ_i}{HI_{add}} \cdot (HQ_i \cdot HQ_j)^{0.5} \quad (5)$$

The normalized WOE for the site-specific mixture, WOE_N, is:

$$WOE_N = \frac{WOE_S}{WOE_{MAX}} \quad (6)$$

The WOE_N is an expression of the strength of the evidence suggesting that interactions may be toxicologically significant relative to the highest possible level of confidence that can be expressed for the site-specific mixture using this method. For example, consider the previously mentioned site-specific mixture with an estimated WOE_S of −0.16 (the sum of interaction factors indicating less-than-additive and greater-than-additive interactions). Suppose the WOE_{MAX} for this site is 0.75. The WOE_N is calculated as −0.16/0.75 = −0.21. Thus, the strength of the available data on the binary interactions, when used with the exposure data from the site, suggests that the net effect of interactions for the mixture is likely to be less-than-additive, as indicated by the minus sign in the WOE_S and WOE_N scores. Relative to (hypothetical) interactions data of the highest possible quality for the same mixture and exposures, overall confidence in the assessment of less-than-additive toxicity for this site-specific mixture is about 20%, as indicated by the magnitude of the WOE_N score (Mumtaz and Durkin 1992; Mumtaz et al. 1994a).

B.2.5. Interactions-Based Hazard Index

Consistent with the suggestion by the NRC (1989) that the hazard index be adjusted for interactions through the application of an uncertainty factor, and with EPA and ATSDR approaches to assessing the noncancer toxicity of individual chemicals, Mumtaz and Durkin (1992) suggest that the hazard index be adjusted for the uncertainty of interactions by the application of an uncertainty factor. The uncertainty factor is modified by the normalized WOE score, WOE_N. The adjustment is performed as follows:

$$HI_I = HI_{add} \times UF_I^{WOE_N} \quad (7)$$

where HI_I is the interactions-based hazard index, HI_{add} is the additivity-based hazard index, and UF_I is an uncertainty factor for interactions. Thus, the hazard index is multiplied by the uncertainty factor for interactions to the power of WOE_N.

The NRC (1989) discussed the use of an uncertainty factor in the range of 1–100 depending on the available interactions information and the concentrations of the components. Mumtaz and Durkin (1992)

note that the value of the uncertainty factor UF_I could be set by taking into account the concern for the magnitude of an interaction, but that suitable data regarding magnitude generally are not available. For the purposes of illustration, an uncertainty factor of 10 has been used in the various examples and exercises performed with this WOE methodology. Because WOE_N can range from -1 (for the highest possible confidence in less-than-additive interactions) to +1 (for the highest possible confidence in greater-than-additive interactions), UF_I to the power of WOE_N can range from 0.1 to 10. The net effect can be to increase *or decrease* the hazard index by a factor of 10. The WOE approach therefore differs from the NRC (1989) approach, which uses an uncertainty factor only to increase the hazard index. It also differs from ATSDR and EPA approaches to assessing the noncancer toxicity of individual chemicals through the derivation of MRLs, RfDs, and RfCs, in which uncertainty factors are applied to make the health criterion more conservative.

As an example of the application of the WOE method, the WOE_N of -0.21 discussed in the previous section and an additivity-based hazard index of 2 are substituted into Equation 7 to estimate the interactions-based hazard index, as follows:

$$HI_I = 2 \cdot 10^{-0.22} = 1.2 \quad (8)$$

For a WOE_N of +0.22, and a hazard index of 2, the interactions-based hazard index would be 3.3. A larger value of WOE_N, +0.75, applied to a hazard index of 2 would result in an interactions-based hazard index of 11.

B.2.6. Strengths and Limitations of the Original WOE Method

The highly prescriptive method for BINWOE classification is designed to encourage a consistent application of the methodology. The application was considered consistent by expert toxicologists who reviewed the results of exercises in which 5–6 teams of toxicologists and risk assessors independently determined BINWOE classifications for the same pairs of chemicals, using the same data (Mumtaz et al. 1994b).

The separation of mechanistic understanding from toxicological significance and equal weighting of these two categories has been questioned on the grounds that mechanistic understanding is important in risk assessment only as it serves to support or modify toxicological significance. Based on analyses of interactions data, the sequence of exposure appears to have a more profound impact on the nature of the interaction than does route or possibly duration (Hertzberg and Durkin 1994). It has been suggested that the sequence of exposure be separated from duration and given a separate weighting factor to better reflect the impact of sequence on the nature of the interaction (Mumtaz and Durkin 1992).

The algorithms do not provide a means for using information on the magnitudes of the interactions for specific pairs of components, should such information be available. Rather, the magnitudes of the interactions among the components of a mixture are represented by a single uncertainty factor, which is modified by the WOE determinations, and then applied to the hazard index. Given the scarcity of suitable data for determining the magnitude of interactions (see Boobis et al. 2011), this may not be a limitation. The normalization process was considered useful as an indicator of confidence in the assessment of direction of interactions for the site-specific mixture and when there is a need to compare scores across hazardous waste sites. It also constrained the value of the interactions-modified uncertainty factor within reasonable limits (0.1–10).

The WOE method (Mumtaz and Durkin 1992; Mumtaz et al. 1994a) has undergone evaluation, and appeared to perform well qualitatively, and quantitatively under some circumstances. The application of the method for deriving BINWOE classifications was considered consistent by expert toxicologists who reviewed the results of exercises in which several teams of toxicologists and risk assessors independently determined BINWOE classifications for the same pairs of chemicals (Mumtaz et al. 1994b). In tests of the WOE method to predict the toxicity of some simple chemical mixtures to animals, BINWOEs for three pairs of chemicals qualitatively predicted whether the results of animal studies would be less-than-additive, additive, or greater-than-additive (Mumtaz et al. 1998). Used with an exponential dose-response model and dose addition to model relative kidney weights, the quantitative WOE method closely predicted the observed dose-response in female rats for intermediate-duration oral exposure to a mixture of four nephrotoxic chemicals with similar MOAs (Mumtaz et al. 1998). The observed dose-response was less than dose additive. The BINWOEs were focused on renal toxicity, and the uncertainty factor used in the algorithm was 10. The WOE method underestimated the relative liver weights in the same animals. The observed dose-response for relative liver weight was slightly greater than dose additive. Thus, the WOE method did not predict toxicity to a target organ that was different from the one for which

the BINWOEs were derived. The WOE method slightly overpredicted the observed dose-response for relative kidney weight in male rats for a mixture of dissimilarly acting nephrotoxins (in female rats, the data variability was so great that the exponential model did not fit the *observed* responses) (Mumtaz et al. 1998). Although these results are suggestive, limitations of this test of the complete WOE method include the substantial variability in the responses of individual animals, small numbers of animals per group, testing of only two dose levels of the mixtures, and lack of rationale for using relative organ weight as an index of toxicity (several other indicators of renal and hepatic toxicity were monitored in the studies that provided the experimental data [Jonker et al. 1993, 1996]).

Subsequent experience with the WOE method revealed, however, that the algorithm does not handle changes in mixture component exposure levels in a reasonable manner. Hertzberg and Teuschler (2002) pointed out that for the conditions involving perfect evidence for synergy (when all $BINWOE_{ij} = 1$), the value of the equation describing the interaction-based hazard index ($HI_{int} = HI \times UF_I^{WOEN}$) becomes constant, regardless of changes in mixture composition. Hertzberg and Teuschler (2002) also noted that the uncertainty factor for interaction (UF_I) works differently than uncertainty factors for RfDs or MRL. Weak data lead to larger uncertainty factors for RfD/MRL development, thereby leading to lower, more public-health-protective, values of estimated safe dose; weak interaction data, in contrast, have minimal influence on hazard index values and thus, do not make the hazard index formula more or less public health protective. ATSDR does not recommend the use of the algorithm and recommends a qualitative WOE approach (Section B.2.2), as suggested by Mumtaz and Durkin (1992).

B.3. MODIFIED WOE METHOD

B.3.1. Modified BINWOE Scores

The modification of the original WOE method that was adopted as part of EPA's mixtures guidance (EPA 2000) employs an alternative WOE classification scheme that focuses on a more integrated interpretation of the data. The suggested numerical weights for the various classifications range from 0 to 1.0 as in the original methodology. As in the original method, two BINWOE determinations are made for each pair: one for the effect of chemical A on the toxicity of chemical B, and the other for the effect of chemical B on the toxicity of chemical A. Unlike the original methodology, less weight is given to less-than-additive interactions under circumstances where there is some uncertainty regarding the interaction (categories II and III). The scheme is shown in Table B-2.

Table B-2. Modified Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions

Default Weighting Factors			
		Direction	
Category	Description	Greater than additive	Less than additive
I.	The interaction has been shown to be relevant to human health effects and the direction of the interaction is unequivocal.	1.0	-1.0
II.	The direction of the interaction has been demonstrated *in vivo* in an appropriate animal model and the relevance to potential human health effects is likely.	0.75	-0.50
III.	An interaction in a particular direction is plausible but the evidence supporting the interaction and its relevance to human health effects is weak.	0.5	0.0
IV.	The assumption of additivity has been demonstrated or is accepted because the information is: A. Insufficient to determine the direction of any potential interaction. B. Insufficient to determine whether any interaction would occur. C. Adequate as evidence that no toxicologic interaction between the components is plausible.	0.0	0.0

Source: EPA 2000

This modified scheme facilitates the integration of toxicological and mechanistic data to support classification in an appropriate category. In common with the original scheme, it encourages the use of structure-activity information to support a classification. Because it is less prescriptive than the original BINWOE classification scheme, the modified scheme may require a greater degree of judgment in actual use.

Like the original method, the modified method does not take dose into account during the BINWOE determination, but rather during application of the algorithms (Section B.3.2).

B.3.2. Modified Interactions-Based Hazard Index

The modified WOE method modifies each component's HQ (where HQ_i is the HQ of the i^{th} component) by the influences of all the other potentially interacting components, resulting in a HQ modified for interactions (HQ_{i_I}). The interactions-modified HQs are then summed to estimate the interactions-based hazard index (HI_I):

$$HQ_{i_I} = \sum_{i \neq j}^{n} HQ_i \, f_{j,i} \, M_{i,j}^{BINWOE_{i,j} \theta_{i,j}} \tag{9}$$

$$HI_I = \sum_{i=1}^{n} HQ_{i_I} \tag{10}$$

The overall process is shown in the following equation (EPA 2000). Some of the terms in Equations 9–11 are modified slightly from those in the cited publications for consistency with the terms used in the original methodology.

$$HI_I = \sum_{i=1}^{n} \left(HQ_i \cdot \sum_{j \neq i}^{n} f_{j,i} \, M_{i,j}^{BINWOE_{i,j} \theta_{i,j}} \right) \tag{11}$$

The term $f_{j,i}$ scales the interactions contribution of chemical *j* by its importance relative to all of the other chemicals interacting with chemical *i*. The toxicological importance is represented by the HQ:

$$f_{j,i} = \frac{HQ_j}{HI_{add} - HQ_i} \tag{12}$$

$M_{i,j}$ is the magnitude of the interaction, defined as an estimate of the maximum effect that chemical *j* has on the threshold or risk-specific dose (e.g., ED_{10}) of chemical *i*. When, as is often the case, data regarding the magnitude are not available, a default value of 5 is used, which is consistent with the upper end of the range of deviation from additivity shown by Smyth et al. (1969). The direction of the interaction is not incorporated into *M*, but rather is part of the term $BINWOE_{i,j}$, which is the BINWOE score. Positive values indicate that the interaction is greater-than-additive, negative values indicate less-than-additive, and the value of zero indicates additivity. $M_{i,j}$, raised to the power of $BINWOE_{i,j} \bullet \theta_{i,j}$, functions as an uncertainty or modifying factor in the estimation of the interactions-based HQs. The term $\theta_{i,j}$ reflects the degree to which components *i* and *j* are present in equitoxic amounts, based on the HQs. This term is incorporated into the algorithm to account for the assumption that the greatest deviation from additivity will occur when both components in a binary mixture are present in equitoxic amounts (EPA 2000). As discussed previously, this assumption is explicit in a model of a deviation from dose additivity proposed

by Finney (1942, 1971). The measure of the deviation from equitoxic amounts is the ratio ($\theta_{i,j}$) of the geometric mean to the arithmetic mean of the HQs:

$$\theta_{i,j} = \frac{(HQ_i \cdot HQ_j)^{0.5}}{(HQ_i + HQ_j)/2} \quad (13)$$

As HQ_i approaches HQ_j, $\theta_{i,j}$ approaches 1, and as HQ_i and HQ_j deviate from each other, $\theta_{i,j}$ approaches 0. Thus, the term $\theta_{i,j}$ reflects how close to equitoxic the two chemicals' doses are. The value for of $\theta_{i,j}$ is the same (0.94) for two components with HQs of 0.01 and 0.02, or 0.1 and 0.2, or 1 and 2.

B.3.3. Strengths and Limitations of the Modified WOE Method

The modified WOE method may require more judgment in the determination of BINWOEs than the original WOE method. The increased flexibility and the integration of toxicological and mechanistic information could lead to a more holistic assessment, but the flexibility also could lead to an erratic application of the methodology. Consistency of application has not been tested.

Although both WOE methods use BINWOE scores to modify an uncertainty (or magnitude) factor that can be based on the magnitude of the interactions, the original method focuses on a single uncertainty factor for the entire mixture, whereas the modified method focuses on individual magnitude factors (*M*) for the effect of each component on the toxicity of each other component. Thus, the potential advantage of the modified WOE method is that information on the magnitude of interactions can be applied directly to the HQ of the chemical whose toxicity is affected. A default magnitude value of 5 is used when data regarding magnitude are not available.

B.4. PRACTICAL CONSIDERATIONS FOR IMPLEMENTATION OF A WOE METHOD IN PUBLIC HEALTH ASSESSMENTS

The number of possible pairs in a mixture of N components is $(N^2-N)/2$. Thus, a mixture of 4 chemicals has 6 possible pairs needing 12 BINWOEs, a mixture of 6 chemicals has 15 possible pairs needing 30 BINWOEs, and a mixture of 9 chemicals has (81-9)/2 = 36 possible pairs needing 72 BINWOEs. Obviously, the practicality of either WOE method may be an issue for mixtures with >4–5 components because of the large numbers of BINWOE determinations that would be required. If an algorithm is used, the calculations are fairly extensive.

Some ways of addressing this issue of practicality are as follows:

- Limit the use of the WOE method to those situations where clarification of the public health hazard is needed, such as sites where exposures to individual components are high enough, relative to health guidelines, that additivity and interactions may result in a significant health hazard.

- Focus the BINWOE effort on chemical pairs that frequently pose the above situation for ATSDR health assessments.

- Make BINWOE determinations available through an easily accessible and readily updated medium, such as the ATSDR website or Interaction Profiles.

- Further develop the patterns approach to analyzing and predicting interactions (Durkin et al. 1995) (see also Appendix A, Section A.3.3) as a potentially cost-effective means of generating BINWOEs.

- Develop a spreadsheet programmed with the appropriate equations to carry out the WOE calculations (if an appropriate algorithm is developed/fully evaluated/selected).

B.5. REFERENCES

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APPENDIX C. METHODS USED OR PROPOSED BY OTHER AGENCIES

C.1. AMERICAN CONFERENCE OF GOVERNMENTAL INDUSTRIAL HYGIENISTS (ACGIH)

ACGIH first discussed its procedure for dealing with exposure to mixtures in 1963 (ACGIH 1984); the procedures have changed little to the present day. ACGIH (2015) recommends additivity approaches for the assessment of occupational hazard to mixtures of chemicals. For mixtures of two or more hazardous agents that act on the same organ system, the ratio of the exposure concentration to the TLV for each component is summed (dose addition, hazard index approach). If the sum exceeds one, then the TLV for the mixture is considered as being exceeded. Exceptions to the hazard index approach can be made when there is good reason to expect that the chief effects of the components are not dose additive (i.e., are independent). According to ACGIH (2015), this can occur when components of the mixture do not have similar toxicological effects or target organs. When the effects are expected to be independent, the TLV for the mixture is exceeded only if at least one component has a HQ that exceeds unity. In effect, the hazard index for the mixture would be the highest HQ for any of the components. (This resembles response addition with completely positive correlation of tolerances, Appendix B.) ACGIH (2015) recommends evaluating synergism or potentiation on a case-by-case basis, and further states that interactions are characteristically exhibited at high concentrations and are less likely at low concentrations.

ACGIH (2015) recommends a special case method for deriving occupational exposure limits for vapors of mixtures of certain refined hydrocarbon solvents containing components that produce acute central nervous system depression and irritation of the eyes and respiratory tract. The method is based on the assumption of dose addition and the mass percent makeup of the following designated groups: C5–C6 alkanes (with the exception of n-hexane), C7–C8 alkanes, C5–C6 cycloalkanes, C7–C8 cycloalkanes, C7–C8 aromatics (with the exception of toluene), C9–C15 alkanes, C9–C15 cycloalkanes, and C9–C15 aromatics (with the exceptions of naphthalene, methylnaphthalene, and indene). More details of the method are detailed in Appendix H of ACGIH (2015) and McKee et al. (2005).

C.2. U.S. OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION (OSHA)

OSHA (1993, 2001) also recommends a hazard index approach that employs the ratio of the exposure concentration to the PEL for each chemical and sums the ratios. If the sum of the ratios exceeds one, then

the exposure limit for the mixture is exceeded. OSHA does not restrict the approach to chemicals with similar effects.

C.3. U.S. NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH (NIOSH)

NIOSH (1976) adopted a similar approach in recommending exposure limits for dichloromethane when carbon monoxide was also present because of the known additivity of the two chemicals with regard to formation of carboxyhemoglobin. NIOSH recommended that the sum of the ratios of each chemical to their recommended exposure limits not exceed one, and that the PELs for dichloromethane be adjusted downward when carbon monoxide levels were >9 ppm in order to keep the sum from exceeding unity. (More recent NIOSH [1992] recommendations are based on carcinogenicity.). In 2004, NIOSH introduced mixtures research agenda, showing the importance of a mixtures program to occupational exposures https://www.cdc.gov/niosh/docs/2005-106/pdfs/2005-106.pdf

C.4. U.S. CONSUMER PRODUCT SAFETY COMMISSION (CPSC)

In response to the U.S. Consumer Product Safety Improvement Act of 2008, the CPSC convened a Chronic Hazard Advisory Panel (CHAP) to study the health effects of all phthalates and phthalate alternatives as used in children's toys and child care articles (CPSC 2014). The panel report concluded that the most sensitive and most extensively studied health effect in animals from phthalates with three to seven or eight carbon atoms in the backbone of the alkyl side chain is referred to as the rat phthalate syndrome (CPSC 2014). In this syndrome, exposing pregnant rat dams causes a syndrome of anti-androgenic effects in male offspring including male reproductive tissue malformations (e.g., hypospadias), retention of nipples/areolae, and reduced anogenital distance depending on dose level and time and duration of exposure. The CPSC (2014) conducted a cumulative risk assessment for five phthalates demonstrated to cause rat phthalate syndrome effects: di(2-ethylhexyl) phthalate, dibutyl phthalate, diisobutyl phthalate, butylbenzyl phthalate, and diisononyl phthalate. The approach used a modified hazard index approach in which estimates of daily intakes of these phthalates were estimated from urine biomonitoring data for phthalate metabolites in individual pregnant women and women of reproductive age in the U.S. NHANES of 2005–2006 and individual children from 2 to 36 months of age in a study called the Study for Future Families (CPSC 2014). The estimates of daily intakes of each of the subject phthalates were divided by a "potency estimate for antiandrogenicity" for the subject phthalate (a value comparable to an RfD derived from a POD in a selected rat study divided by uncertainty factors) to derive a HQ. The hazard index for each individual subject was derived by summing the HQs for the subject phthalates. The distributions of the hazard indices for these phthalates in pregnant women and

children were assessed: about 10% of the studied pregnant women and about 5% of studied mothers and children had hazard indices >1 (CPSC 2014).

C.5. EPA OFFICE OF RESEARCH AND DEVELOPMENT (ORD)

The EPA ORD (EPA 1986) guidelines for risk assessment of chemical mixtures recommended the use of exposure and health effects data for the mixture of concern or a similar mixture if available. If not, the use of data for the components was recommended. When more than one of these approaches is feasible, EPA (1986) recommended a comparison of results from the different approaches.

The guidelines recommended the assessment of interactions data, when available, in terms of relevance to subchronic or chronic exposure and suitability for quantitatively altering the risk assessment. Interactions data were considered likely to be available mainly for pairs of chemicals, which could be assessed separately from those with no such information. The guidelines recommended, however, exploring the possibility that other components of the mixture may interfere with the interaction of the chemical pair on which quantitative interaction data are available. If interference appears likely, then quantitative alteration of the risk assessment may not be justifiable.

The assessment of the noncarcinogenic effects of the components usually proceeds by the hazard index method. Because it assumes dose additivity, the hazard index method is most suitable for chemicals with similar effects. If the mixture includes chemicals that have different effects, then EPA recommended the calculation of separate hazard indices for each end point of concern. The guidelines mentioned that if data are sufficient to derive individual acceptable levels for a spectrum of effects, the hazard index may suggest what types of effects might be expected from the mixture exposure. Subsequent guidance for Superfund risk assessment gave further explicit directions for the hazard index approach, including the combining of hazard indices for multi-route exposure and the calculation of separate hazard indices for different target organ toxicities (EPA 1989a). For carcinogenic effects, the guidelines recommended summing the risks across carcinogenic components (i.e., assume response addition).

EPA ORD developed additional mixtures guidance for risk assessment (EPA 2000), which supplemented, but did not replace, the broad principles and concepts in the original EPA ORD guidelines (EPA 1986). The supplementation emphasized an interactive and iterative problem formulation step to supplement the four parts of the EPA paradigm for assessing human health risks applied to chemical mixtures: hazard identification, dose-response assessment, exposure assessment, and risk characterization. The recommended problem formulation process involved the following three steps: (1) evaluate the nature of

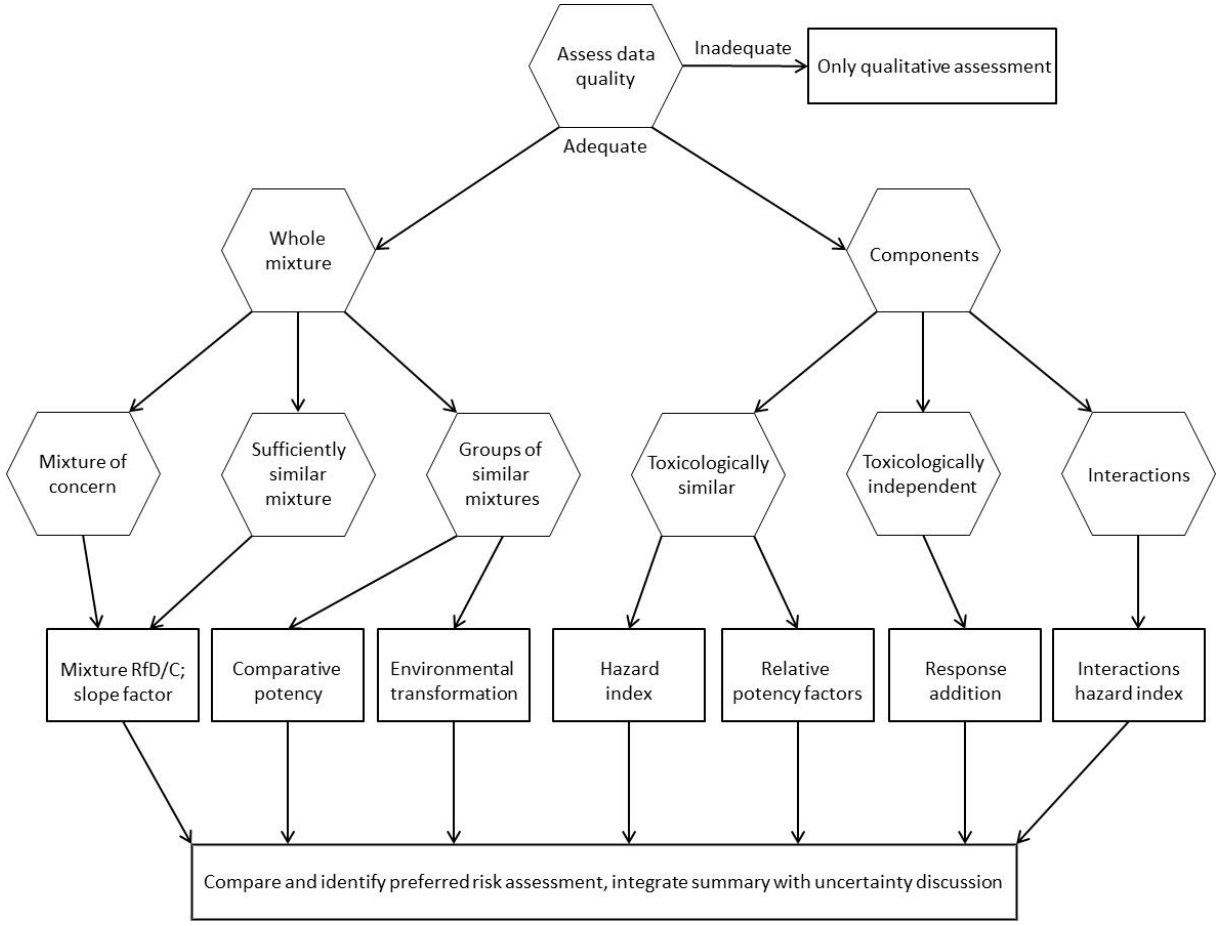
the problem; (2) define the objectives of the risk assessment; and (3) develop a data analysis and risk characterization plan. The problem formulation should: (1) assess the quality, quantity and pertinence of available information, (2) select end points to be assessed; and (3) review conceptual models that describe the relationships between exposure to the mixtures and risk for adverse health outcomes. Figure C-1 is presented to illustrate the different types of risk assessment processes that could be implemented based on the availability and quality of the data for the mixture of concern. EPA (2000) recommended that all possible assessment paths should be performed.

The guidance focused on procedures for dose-response assessment and risk characterization, noting that other EPA guidance existed to address exposure assessment and specific toxic end point evaluations. The supplemental guidance provided descriptions of methods for using whole-mixture data on a toxicologically similar mixture, methods on incorporating information on toxicologic interactions to modify a hazard index, and generalized procedures for mixtures involving classes of similar chemicals (EPA 2000). Expanded discussion was also included of concerns and uncertainties to be considered when using only whole-mixtures data (e.g., environmental transformations) or only data on the individual chemical components (e.g., the possible existence of interactions among the components—i.e., deviations from additivity).

C.6. EPA OFFICE OF AIR AND RADIATION (OAR)

The EPA OAR has completed four National Air Toxic Assessments (NATAs) for data collected in 1996, 1999, 2002, and 2005. The NATAs estimate chronic cancer risk and noncancer hazard from inhaling chemicals identified as air toxics (see EPA 2013). The assessments are based on collected ambient air concentration data for air toxics from stationary sources (e.g., large industrial facilities and smaller sources such as gasoline stations), mobile sources (e.g., cars and trucks), background sources (e.g., natural emission sources) and secondary formation (i.e., pollutants formed from other pollutants emitted in air) across broad geographic areas in the United States (e.g., counties, states). In the latest NATA, data for 177 air toxics plus diesel exhaust particulate matter were collected in 2005 and risk estimates of cancer or noncancer effects were developed for a subset of 139 chemicals with health data based on chronic exposure (cancer results for 80 air toxics and noncancer results for 110 air toxics) (EPA 2011c). Exposures were estimated from atmospheric dispersion models and human activity pattern data. Cancer risks for individual carcinogenic air toxics in outdoor air were calculated by multiplying estimates of chronic lifetime exposure levels by upper-bound inhalation unit risk estimates. Individual cancer risks

Figure C-1. EPA (2000) Description of Different Risk Assessment Paths for Chemical Mixtures Based on the Availability and Quality of the Data



EPA = Environmental Protection Agency; RfD/C = reference dose/concentration

Source: EPA 2000

were added under the assumption of response addition (independent action) to estimate cumulative cancer risks. When the risks from all carcinogenic air toxics were added, the approach estimated that there were about 3,100 census tracts (5% of about 66,000 census tracts in the United States) with increased cancer risks greater than 100 in a million. A target-organ, dose-additivity hazard index approach was used to estimate hazard potential for noncancer respiratory and neurological effects. Adequate toxicity data were available to calculate respiratory-effects HQs for 41 air toxics. The cumulative respiratory hazard indices for U.S. census tracts indicated that about 69 million people (of about 285 million U.S. residents) lived in regions with respiratory hazard indices >1.0 and about 174,000 people lived in regions with respiratory hazard indices >10. The EPA emphasized that the "NATA is a prioritization tool to identify geographic areas, pollutants and emission sources that should be evaluated further to gain a better understanding of health risks posed by air toxics."

C.7. EPA RISK ASSESSMENT FORUM

The EPA Risk Assessment Forum (EPA 2003) described a framework for conducting cumulative risk assessment, defined as "an analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors." The framework does not limit "agents or stressors" to only chemicals, but includes other biological or physical agents or conditions, and specifies that "combined risk" does not mean that risks are necessarily added, but rather that some analysis should be conducted to assess how the targeted agents or stressors may interact." Three phases to the framework were described: (1) planning, scoping, and problem formulation; (2) analysis; and (3) risk characterization. The first phase entails the establishment of the goals, breadth, depth, and focus of the assessment and the production of a conceptual model that establishes the stressors to be evaluated, the health or environmental effects to be evaluated, and what is known about exposure-response relationships for the subject agents or stressors. The analysis phase includes developing exposure profiles, considering potential interactions among agents or stressors, and estimating risks to the population or populations under consideration. The end product of phase 2 is an analysis of the risks associated with the multiple agents or stressors to which the studied population or populations are exposed. The third phase evaluates the significance of the risk estimates, the reliability of the estimates and associated uncertainties, and the overall confidence in the assessment. Discussion of other details of this framework were previously discussed in Section 3.3.8 of this document.

C.8. EPA OPP

The EPA OPP is required under legislative statutes to determine with reasonable certainty that consumption of raw agricultural commodities or processed foods containing residues of a specific pesticide will not cause harm to humans, especially infants and children (EPA 2002b). The FQPA of 1996 further requires EPA to: (1) base its risk assessment for each pesticide chemical on aggregate exposure (total food, drinking water, residential, and other nonoccupational exposures); (2) consider available evidence concerning the cumulative effect on infants and children of pesticide chemicals having a common mechanism of toxicity; and (3) use an additional 10-fold margin of safety to take into account potential pre- and postnatal toxicity and completeness of the toxicity and exposure databases (EPA 2002a). This additional safety factor is often referred to as the FQPA Safety Factor.

As described earlier, the EPA OPP approach for cumulative risk assessments for pesticides sharing a common effect through a common mechanism involves: (1) determination of whether or not a group of structurally related pesticides produces a common effect by a common mechanism; (2) selection of an index chemical and determination of RPFs for members of the group; (3) determination of concentrations of member chemicals in foods and environmental media; (4) estimation of intakes for target population for multiple exposure pathways using exposure models; and (5) assessment of risks for target populations using a POD/MOE hazard indicator method when appropriate data are available (EPA 2002b).

The EPA OPP cumulative risk assessment for N-methyl carbamates provides an illustrative example of the approach. EPA (2007b) determined that N-methyl carbamate insecticides represent a common mechanism group based on similar structural characteristics and shared ability to produce neurological effects via inhibition of acetylcholine esterase (AChE) at the active enzymatic site. A multi-chemical, multi-pathway PBPK/PD model could not be developed for the cumulative risk assessment, because appropriate pharmacokinetic data for model development were only available for one carbamate insecticide, carbaryl. Based on an analysis of available data, including data collected by EPA and data submitted for registration, acute AChE inhibition, measured at the peak time of effect in rats, was determined to be the most sensitive effect from exposure to carbamates and thus, the pertinent effect of concern. A component-based RPF approach, assuming dose additivity, was used in the cumulative risk assessment. RPFs for 10 carbamates (and several carbamate metabolites—aldicarb sulfone, aldicarb sulfoxide, and 3- and 5-hydroxycarbofuran) were developed based on brain AChE inhibition data for adult rats (Table 6). The rat brain AChE inhibition data were modeled with a dose-time response model to estimate BMD_{10} values (doses at which AChE was inhibited by 10%), and the RPF values were

calculated by dividing the BMD_{10} value for the subject carbamate by the BMD_{10} value for the index carbamate, oxamyl. Oxamyl was chosen as the index chemical, because oxamyl, compared with the other nine carbamates, had the most robust data base for all three pertinent routes of exposure (oral, dermal, inhalation).

Uncertainty and extrapolation factors were incorporated into the cumulative risk assessment in two ways:

1. Adjustment of the RPF: Chemical-specific information was evaluated, when available, to determine chemical-specific inter-species uncertainty factors (animal to human extrapolation) and FQPA factors to arrive at adjusted RPF values for children and adults (Table 7). Chemical-specific FQPA factors were calculated, when appropriate data were available, by dividing an adult BMD_{10} by a pup BMD_{10} for AChE inhibition. In the absence of appropriate data, the default FQPA safety factor value of 10 was used. Chemical-specific interspecies uncertainty factors were calculated similarly when appropriate data were available to compare human BMD_{10} values for AChE inhibition with rat BMD_{10} values. In the absence of appropriate data, a default interspecies uncertainty factor of 10 was used (see Table 7).

2. Incorporation into the target MOE: A default uncertainty factor of 10 for intrahuman variability was taken as the target MOE for each of the carbamate insecticides. The PODs, used in the cumulative risk assessments to compare against exposure-based TEQs estimates were the route-specific rat $BMDL_{10}$ values for brain AChE inhibition shown in Table 8.

Table C-1. EPA RPFs for Oral, Dermal, and Inhalation Exposure to Carbamate Insecticides Based on Rat Brain Acetylcholinesterase Inhibition

Chemical	Oral RPF	Dermal RPF	Inhalation RPF
Aldicarb	4	ND[b]	ND
Aldicarb sulfone[a]	3.44	ND	ND
Aldicarb sulfoxide[a]	3.68	ND	ND
Carbaryl	0.15	0.71	0.51
Carbofuran	2.4	ND	ND
3- and 5-Hydroxycarbofuran[a]	2.4	ND	ND
Formetanate hydrochloride	2.18	ND	ND
Methiocarb	0.18	0.09	0.62
Methomyl	0.67	ND	ND
Oxamyl	1	1	1
Primicarb	0.02	ND	ND
Propoxur	0.11	0.03	0.18
Thiodicarb	0.89	ND	ND

[a]Values for aldicarb sulfone and aldicarb sulfoxide were calculated based on molecular weight conversions from aldicarb assuming equipotency to aldicarb. 3- and 5-Hydroxycarbofuran were assumed to be equipotent to carbofuran.
[b]ND = not derived due to lack of data.

EPA = Environmental Protection Agency; RPF = relative potency factor

Source: EPA 2007b

Table C-2. EPA Adjusted Oral RPFs for Children and Adults Exposed to N-Methyl Carbamates Based on Interspecies and FQPA Factors

Chemical	Oral RPF	Interspecies factor	FQPA factor for children	Adjusted RPF for children	Adjusted RPF for adults
Aldicarb	4	2	2	16	8
Aldicarb sulfone	3.44	2	2	13.8	6.9
Aldicarb sulfoxide	3.68	2	2	14.7	7.4
Carbaryl	0.15	10	1.8	2.7	1.5
Carbofuran	2.4	10	2.75	66	24
3- and 5-Hydroxycarbofuran	2.4	10	2.75	66	24
Formetanate hydrochloride	2.18	10	2.03	44	22
Methiocarb	0.18	10	10	18	1.8
Methomyl	0.67	5	3.05	10	3.3
Oxamyl	1	3	3.48	10	3
Primicarb	0.02	10	10	2	0.2
Propoxur	0.11	10	10	11	1.1
Thiodicarb	0.89	10	10	89	8.9

EPA = Environmental Protection Agency; FQPA = Food Quality and Protection Act; RPF = relative potency factor

Source: EPA 2007b

Table C-3. Oral, Dermal, and Inhalation BMD_{10} and $BMDL_{10}$ Values for Rat Brain Acetylcholinesterase Inhibition by Oxamyl, the Index Chemical for the EPA Cumulative Risk Assessment for N-Methyl Carbamates

End point	Oral	Dermal	Inhalation
BMD_{10}	0.24 mg/kg	34.91 mg/kg	0.0047 mg/L
$BMDL_{10}$	0.18 mg/kg	17.05 mg/kg	0.0038 mg/L (converted to 0.66 mg/kg)

BMD = benchmark dose; BMDL = benchmark dose limit; EPA = Environmental Protection Agency

Source: EPA 2007b

EPA (2007b) conducted route-specific cumulative risk assessments for adult and children exposures to N-methyl carbamate insecticides by incorporating the RPF values into an MOE approach applied to food, water, and residential exposure pathways. The residential pathways comprised oral, dermal, and inhalation exposures. Concentrations of carbamate residues in appropriate media (e.g., food, drinking water) were multiplied by appropriate interspecies- and FQPA-adjusted RPF values (Table 7) and summed to arrive at oxamyl-equivalent concentrations (TEQs), which were then used in exposure models to estimate oxamyl equivalent intakes (in units of mg/kg body weight) for appropriate exposure scenarios

for groups of adults and children in the general population. MOE values were calculated by dividing the appropriate oxamyl POD (e.g., the oral rat $BMDL_{10}$—Table 8—for oral exposure scenarios) by the estimated oxamyl TEQ intake. MOE values <10 were taken as values requiring some mitigation action; those >10 were assessed to be without the need for mitigation. EPA (2007b) determined total MOE values for combined estimates of food, water, and residential exposure scenarios (i.e., aggregate exposure), showing with a sensitivity analysis that the food pathway was the dominant exposure pathway for the general population.

C.9. U.S. NATIONAL ACADEMY OF SCIENCE (NAS)/NATIONAL RESEARCH COUNCIL (NRC)

In 1972, at the request of the EPA, the NAS recommended health-based stream criteria for a large number of pollutants. A component of this appraisal was multiple chemical exposure (NAS 1974). The NAS recommended a hazard index approach, whereby the sum of the ratios of the measured concentrations to the acceptable concentrations for the components was to be kept at a level equal to or lower than unity.

In 1989, at the request of EPA, The Safe Drinking Water Committee of the NRC suggested possible modifications of the then-current approaches for estimating the toxicity of mixtures in drinking water (NRC 1989). The NRC suggested that mixture components be grouped by end point, such as specific organ toxicity and carcinogenicity in order to assess their combined risk or hazard.

For noncancer end points, the NRC (1989) suggested a modified hazard index that sums similar toxicities and an uncertainty factor for possible synergism, depending on the information regarding interactions and the concentrations of the components. The uncertainty factor could range from one to 100. If information regarding potential interactions is available and suggests that interactions are not likely, or if the concentrations are low, the uncertainty factor could be set at one. The NRC also suggested that separate hazard indices be calculated for each toxic end point, including those that occur at higher exposure levels than the end point that is the basis for the acceptable exposure level for a component. A weighting factor would be applied to account for the lesser sensitivity of the other end points, unless an acceptable exposure level for the other end points was available. The method is similar to the TTD modification of the hazard index method, discussed previously, except that the NRC further suggested summing the hazard indices across all toxic end points.

For carcinogenic end points, the NRC (1989) concluded that it was appropriate to sum the risks (response addition with completely negative correlation of tolerances) for low-dose exposure to a mixture of carcinogens (doses with relative risks of <1.01).

The NRC (2004a) report, *Air Quality Management in the United States*, recommended that the EPA address multiple pollutants in its National Ambient Air Quality Standards (NAAQS) review and standard setting process. However, the committee making this recommendation did not "believe that the science has evolved to a sufficient extent to permit the development of multipollutant NAAQS, it would be scientifically prudent to begin to review and develop NAAQS for related pollutants in parallel and simultaneously." In response to this recommendation, EPA convened a public workshop in 2011 to discuss scientific issues and data gaps related to adopting multipollutant science and risk assessment approaches for priority hazardous air pollutants identified by the EPA under the Clean Air Act (Johns et al. 2012). The major conclusion from the workshop called for the development and adoption of a framework and methods for conducting multipollutant science and risk assessments of the well-studied priority air pollutants (Johns et al. 2012).

The NRC (2008) report, *Phthalates and Cumulative Risk Assessment: Tasks Ahead*, recommended that the EPA should conduct a cumulative risk assessment for phthalates using the dose-addition concept to all phthalates that have anti-androgenic activity. This recommendation was based on studies examining effects on developing male reproductive end points in rats after oral exposures to mixtures of phthalates or phthalates plus other anti-androgenic compounds showing that dose-addition models provided adequate fit to observed dose-response data (Christiansen et al. 2009; Hass et al. 2007; Howdeshell et al. 2007, 2008; Rider et al. 2008; see Section 3.3.1.2 *Evidence to Support or Refute the Use of Default Dose-Additivity Approaches*). To date, EPA has not conducted a cumulative risk assessment for phthalates or other anti-androgenic chemicals, but CPSC (2014) published a cumulative risk assessment for anti-androgenic effects from five phthalates, using a modified hazard index approach (see Section C.4).

C.10. U.S. DEPARTMENT OF ENERGY (DOE)

To estimate potential health effects to workers and the public exposed to unplanned release of mixtures of chemicals, the U.S. DOE followed the principles of the EPA (1986, 2000) chemical mixtures guidelines to establish a chemical mixtures methodology using a dose-additive hazard index approach (Yu et al. 2010, 2013). In this method, a hazard index (comparable to EPA's HQ) for each chemical in the mixture is calculated that is the ratio of the exposure concentration for the component "at a given receptor site" to

the toxicity guidance value for the component. Exposure concentrations are estimated with Gaussian atmospheric dispersion models that calculate exposure concentrations based on the amount of each chemical in the mixture available for release to the atmosphere, the manner of release (e.g., spill or explosion), the chemical and physical properties of each chemical, the time within the release plume, release event parameters, and meteorological conditions. The default, screening-level approach uses Protective Action Criteria (PACs) that are determined from (in order of priority) Acute Exposure Guideline Level (AEGL) values, Emergency Response Planning Guideline (ERPG) values or Temporary Emergency Exposure Limit (TEEL) values. Different PAC benchmark values are established to indicate threshold concentrations for increasing severity of acute effects (i.e., levels below the threshold value are not expected to produce effects of the indicated severity): PAC-0, threshold for no adverse effects; PAC-1 threshold for mild or transient effects; PAC-2, threshold for irreversible or serious effect that could impair a person's ability to take protective actions; and PAC-3: threshold for life-threatening effects. As an initial screen to provide protection for first responders using this method, a cumulative hazard index is calculated by summing individual component hazard indices using PAC-1 or PAC-2 values for all known components in the mixtures, regardless of their target organ or expected MOA; cumulative hazard indices >1 indicate risks from acute exposure to the mixture and the need to take some mitigating action. A refinement to the screening level cumulative hazard index approach is analogous to the TTD method described in Section 3.3.3 of this document, in which cumulative hazard indices for chemicals in the mixture producing the same or similar effects are calculated. All chemicals in the DOE database with PACs (approximately 3,300 chemicals) are assigned any number of 60 health code numbers as guided by available toxicity data for the individual chemical (see Table 1 in Yu et al. 2013). Some of the health code numbers are for acute effects, others are for chronic effects. The health code numbers are used to group individual hazard indices for chemicals in the mixture of concern affecting the same or similar target organs, which are then used to calculate cumulative hazard indices for specific target organs or effects. The approach assumes that acute and chronic effects are independent and for a specific target organ calculates separate cumulative hazard indices for acute and chronic effects. For example, separate cumulative hazard indices are calculated for acute reproductive effects (health code number, 5.00) and chronic reproductive effects (health code number, 5.11). Yu et al. (2013) reported that activities were ongoing to evaluate modifications to the target organ/effect hazard index approach. The modifications under consideration involved the development of weighting factors for target organ hazard indices based on rankings of health code numbers for each type of PAC value.

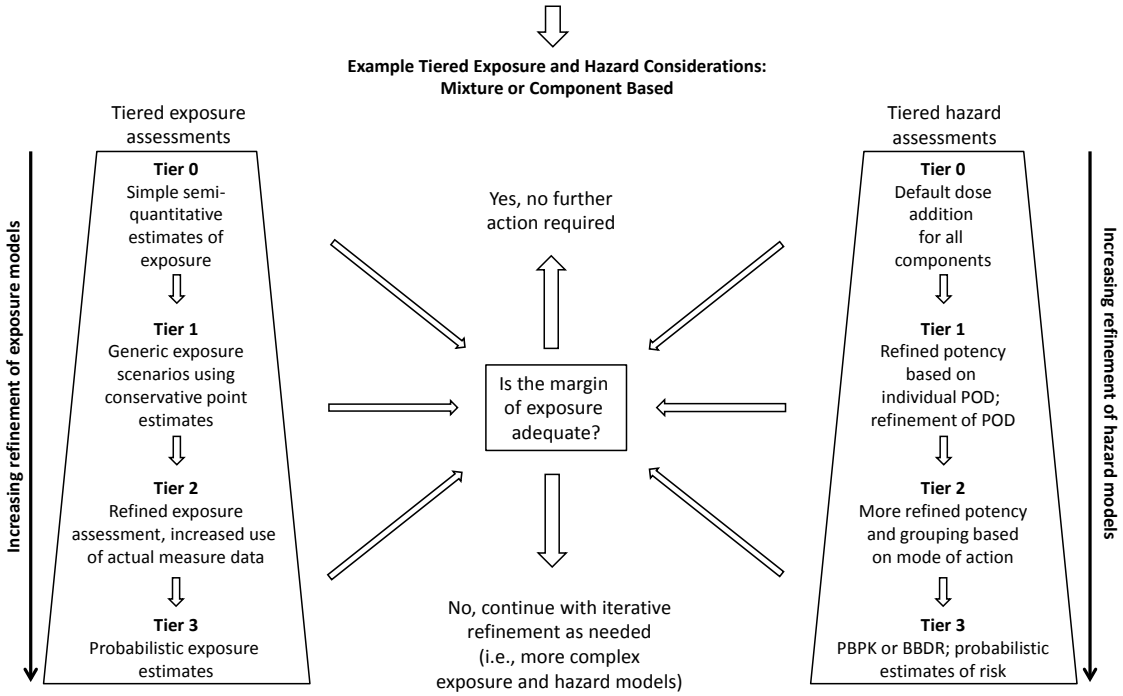
C.11. WORLD HEALTH ORGANIZATION (WHO)/INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY (IPCS)

Based on a workshop convened by the WHO/IPCS, a framework for assessing health risks from combined exposure to multiple chemicals has been described (Meek 2013; Meek et al. 2011). The framework entails tiered parallel assessments of exposure and hazard that increase in refinement and data depth and are accompanied by an iterative problem formulation process that asks key questions about the nature and likelihood of exposure to multiple chemicals and the rationale for considering multiple chemicals in an assessment group (Figure C-2). Within each tier of assessment, information on exposure and hazard from the mixture are brought together to assess the MOE (the ratio of some POD assessment (NOAEL/LOAEL or BMD_{10}) of combined toxicity to an estimate of exposure). From this MOE assessment, decisions are made about continuing (or not) with iterative refinements based on the availability of appropriate data and scope of the assessment. Tier 0 of the framework calls for simple semi-quantitative estimates of exposure and a default dose-addition hazard approach for all components of the mixture. Tier 1 calls for refined exposure scenarios using conservative point estimates and refined PODs. Tier 2 calls for increased use of actual data in the exposure assessment and more refined potency and groupings based on MOA. Tier 3 calls for probabilistic exposure estimates and refined probabilistic estimates of hazard risk involving PBPK and PBPK/PD modeling when appropriate. The tiered approach allows consideration of the efficiency of use of resources: each progressive tier is more refined, requiring more labor and data (Meek 2013).

A case study applied the WHO/IPCS framework to conduct a screening-level assessment under the Canadian Environmental Protection Act of a group of seven polybrominated diphenyl ethers (PBDEs) present in commercial mixtures used as flame retardants in a number of consumer products (Meek et al. 2011). In the Tier 0 assessment, semi-quantitative measures of exposure was determined through comparison of relative rankings, physicochemical properties, and use patterns with those for congeners with deterministic estimates of exposure. In the absence of toxicity guidance values for the individual congeners, a hazard index could not be calculated, but the summation of semiquantitative estimates of exposure was greater than the LOAEL for the most toxic congener with toxicity data. This simplified MOE was used to prompt a Tier 1 assessment. In the Tier 1 assessment, upper-bounding estimates of total intakes of PBDEs were developed based on maximum levels in air, water, dust, foods, and human breast milk and reference intake values for six age groups within the Canadian population. Based on review of available results from animal toxicity tests with several of the individual congeners or commercial PBDE mixtures identifying effects on liver, thyroid, and neurological (behavioral) development in neonatal mice, a LOAEL of 0.8 mg/kg body weight/day for effects on locomotion,

rearing, and total activity was selected as the critical (most sensitive) POD. A MOE of about 300 was calculated based on a comparison of this POD with the upper bounding intake estimate of 0.0026 mg/kg body weight/day. Descriptions and considerations of the uncertainties in the Tier 1 exposure and dose-response assessments were used to make recommendations for additional risk assessment and research activities. Assessments at the Tier 2 or 3 levels were not conducted (Meek et al. 2011).

Figure C-2. World Health Organization/International Programme on Chemical Safety Framework for Evaluating the Risk of Combined Exposure to Multiple Chemicals



BBDR = biologically based dose-response; PBPK = physiologically based pharmacokinetic; POD = point of departure

Source: Meek et al. 2011

C.12. HEALTH COUNCIL OF NETHERLANDS

The Health Council of Netherlands published a report outlining a decision flow chart to guide hazard identification and risk assessments for chemical mixtures (Figure C-3). The approach was developed to guide hazard identification and risk assessment for specified combinations of chemicals, as well as for more complex mixtures that may have components that are unknown. The flow diagram recommended using toxicity data on the mixture of concern or a "comparable mixture" if data are available, and using toxicity data on constituents (i.e., components) if toxicity data for the mixture itself or a comparable mixture are not available. For the component-based approaches, the decision tree called for determining whether or not few components or many components were present in the mixture.

For the case of many components, a step was recommended to decide whether or not a few components of most concern (based on a combination of severity of hazard and concentration in the mixture) should be used to estimate toxicity of the mixture, or a grouping or lumping approach such as that described by Verhaar et al. (1997) should be used. Continuing in the component-based path, the approach called for grouping chemicals with similar toxicological action, assessing information on possible interactions and using dose addition approaches for assessing risks. For components with dissimilar toxicological action, a response-addition approach was recommended, using exposure limits for individual agents for risk assessment. To evaluate evidence for interactions among components of the mixture, the approaches described by Mumtaz and Durkin (1992) and EPA (2000) were recommended.

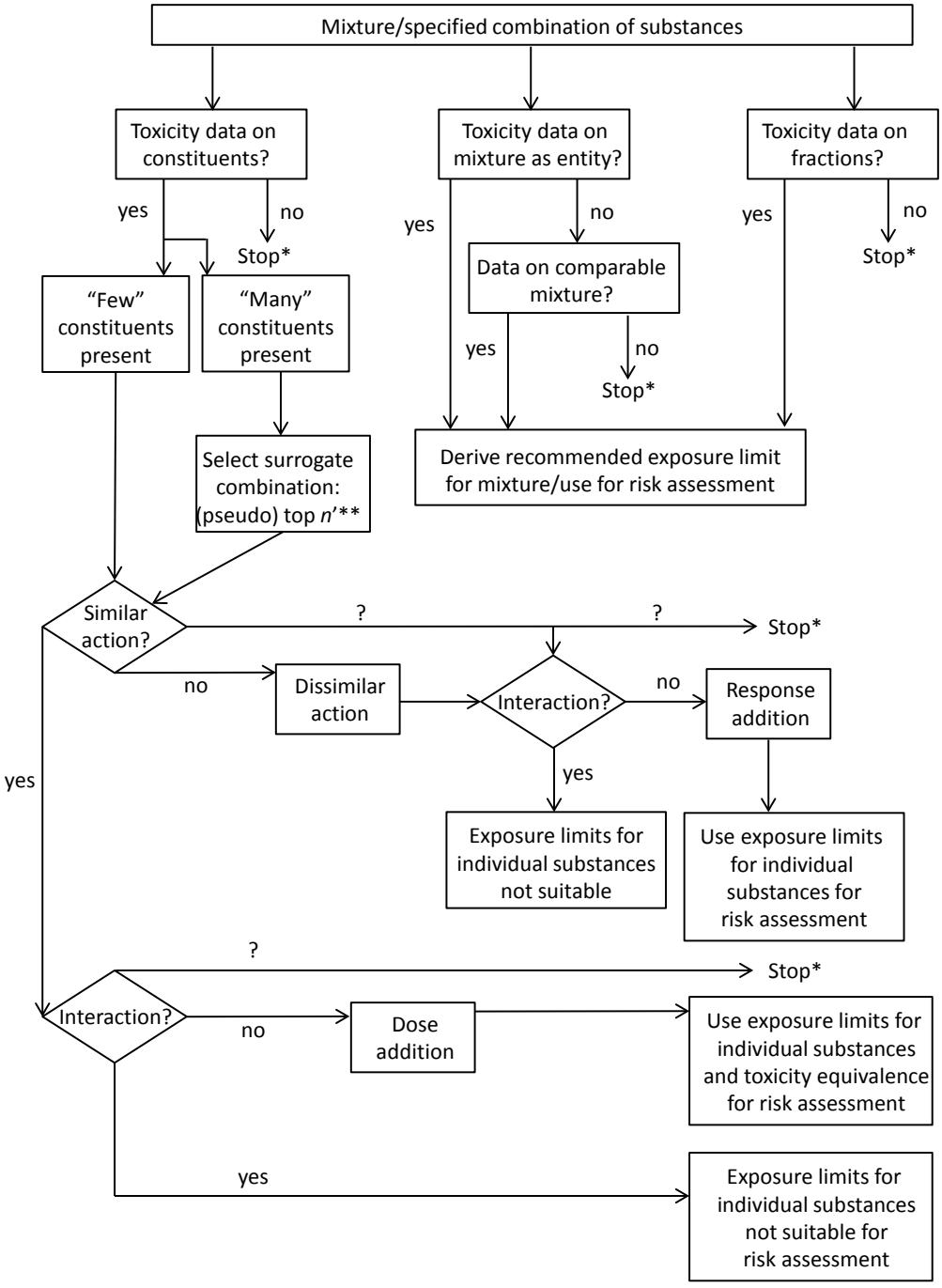
C.13. NORWEGIAN SCIENTIFIC COMMITTEE FOR FOOD SAFETY

The Norwegian Scientific Committee for Food Safety (2013) published a report, *Combined Toxic Effects of Multiple Chemical Exposures*, outlining a decision-tree flow chart for use in human health risk assessments of chemical mixtures or concurrent exposure. The flow chart did not suggest approaches for the cases where toxicity data may exist for the mixture of concern or a similar mixture. Two key opinions incorporated into the flow chart, which represents a component-based approach (see Figure C-4), are as follows:

1. For chemicals with similar MOAs, adverse effects from multiple exposures occur due to dose addition, even if exposures to components are below their respective acceptable or tolerable daily intakes.

2. For chemicals with dissimilar MOAs, adverse effects from multiple exposures are not expected when the exposures to the individual components are below the respective acceptable or tolerable daily intakes. When compounds in the mixture are thought to act independently of each other, the recommended approach is a chemical-specific approach for each component, if pertinent toxicity data are available.

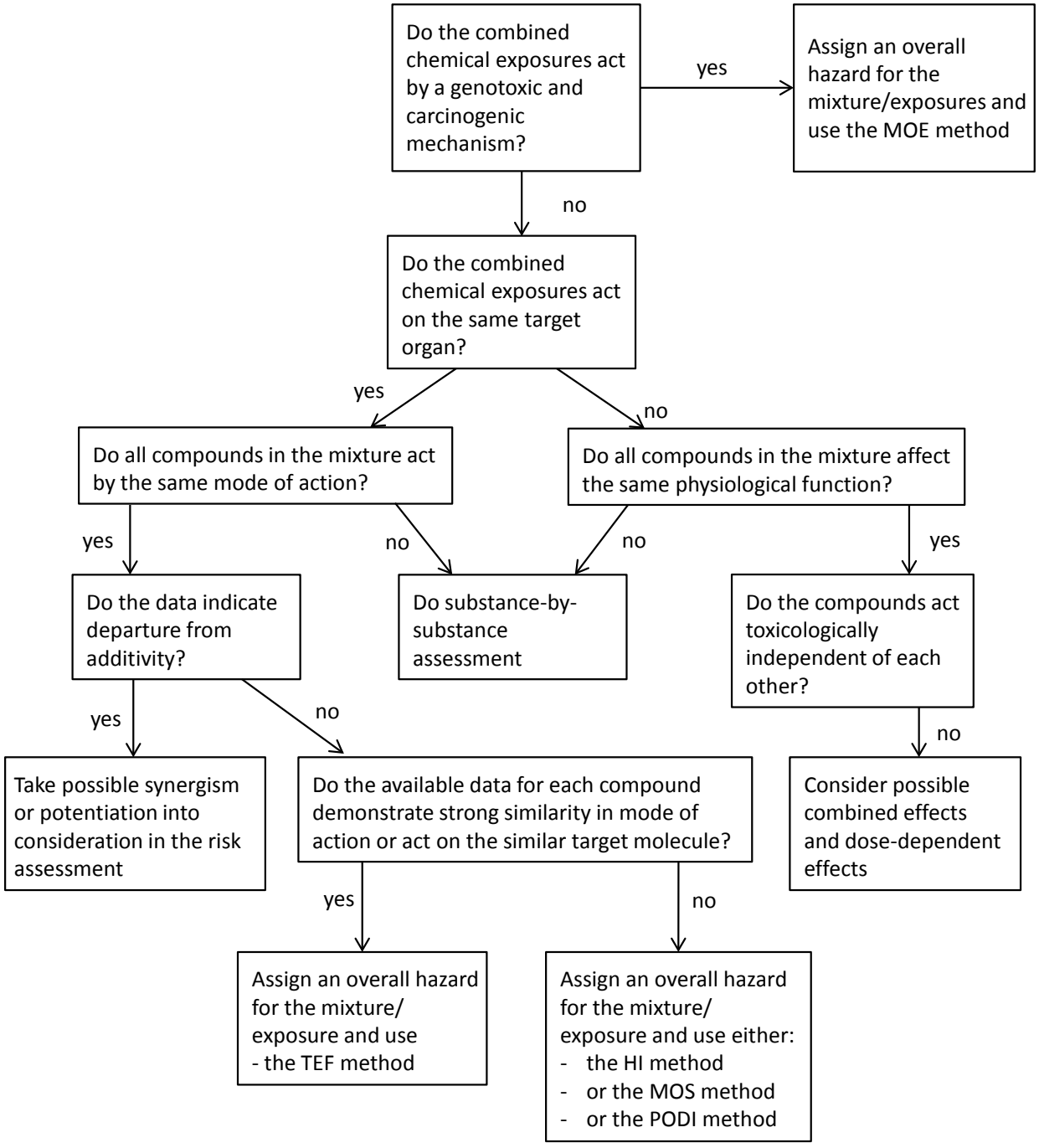
Figure C-3. Health Council of Netherlands Framework for Evaluating the Risk of Combined Exposure to Multiple Chemicals



*Stop = data required to complete the evaluation.
**"Top *n*" and "pseudo top *n*;" *n* represents the most "risky" chemicals or groups of chemicals, respectively.

Source: Feron et al. 2004 (Reprinted from Environmental Toxicology and Pharmacology, 18(3):215-222. Copyright (2004), with permission from Elsevier)

Figure C-4. Norwegian Scientific Committee for Food Safety Flow Chart for Use in Risk Assessments of Multiple Chemical Exposures



HI = hazard index; MOE = margin of exposure; MOS = margin of safety; PODI = point of departure index; TEF = toxic equivalency factor

Source: Norwegian Scientific Committee for Food Safety 2013

C.14. DANISH MINISTRY OF THE ENVIRONMENT

The Danish Ministry of the Environment (DEPA 2009) convened a workshop to examine existing scientific knowledge on combination effects of endocrine disrupters, with a focus on regulatory aspects. Several consensus recommendations were made by workshop participants:

1. "Cumulative risk assessment for endocrine disrupters was seen as both necessary and feasible. The predominant chemical-by-chemical approach in risk assessment was regarded as insufficiently protective against the possibility of mixture effects/ effects of combined exposure.

2. The application of dose (or concentration) addition as an assessment method was recommended as a default, until evidence as to the suitability of alternative assessment concepts emerges.

3. A pre-occupation with mechanisms or modes of action as the starting point for the grouping of endocrine disrupters into classes to be subjected to mixtures risk assessment was seen as not practical and scientifically hard to justify. Instead, grouping criteria should focus on common health related effects and the likelihood of co-exposures.

4. The full potential of cumulative risk assessment for endocrine disrupters cannot be reached without filling a number of data gaps, most importantly in the area of mixtures exposure assessment.

5. An enhancement of the legal framework in Europe with a view to mandating cumulative risk assessment should be given serious consideration."

C.15. EUROPEAN FOOD SAFETY AUTHORITY

The European Food Safety Authority's Panel on Plant Protection Products and their Residues evaluated existing methodologies for assessing risks of exposure to two or more pesticides in combination and made recommendations for refining the methodologies (EFSA 2008). The panel noted that their recommendations were for component-based approaches for groups of pesticides producing common adverse outcomes or MOAs. For mixtures of pesticides with different targets or MOAs, the panel concluded that there was no evidence for dose additivity at low exposure levels below toxicological reference values, and that risk associated with such mixtures are determined by the component with the highest HQ. This conclusion is consistent with the concept of response addition (i.e., independent action) for these cases. The panel also concluded that no more than dose additive effects were expected for pesticides with a common target or MOA, noting that: (1) pesticide residues are generally below individual NOAELs and (2) "although toxic interactions from pesticide residues cannot be ruled out, there

is no empirical evidence for their occurrence at the expected levels of exposure from pesticide residues in food." The recommendations call for:

1. A tiered parallel assessment of exposure and hazard, conducting iterative, increasingly complex and data-intensive risk assessments by MOE comparisons between exposure and hazard estimates using increasingly data intensive methods (i.e., methods of increasing refinement).

2. Initially identifying the common assessment group "in broad terms," evaluating the evidence for common adverse outcomes or MOAs, and refining the group with more refined assessments of hazard.

3. Dose-additive, hazard index approaches, initially for all components or components with common effects and proceeding through more refined approaches involving RPFs derived from NOAELs or BMDs and, finally, RPFs refined by PBPK/PD models, if appropriate models are available.

4. Tiered exposure assessments, initially using deterministic modeling approaches, proceeding through probabilistic modeling approaches if appropriate data are available.

5. Recognition and articulation of uncertainties in both the exposure and hazard assessment portions of the risk assessment, noting a qualitative scheme for evaluating sources of uncertainties that may cause small, medium, or large, over- or under-estimation of risk. The panel noted that sources with large uncertainty potential warrant sensitivity analysis and "provide the greatest scope for refinement of the assessment."

In a related Scientific Opinion, the Panel on Plant Protection Products and their Residues evaluated existing methods for assessing chemicals acting by dissimilar MOAs and recommended dose-addition approaches for the assessment of multiple pesticides with dissimilar MOAs, provided that they produce a common adverse outcome (EFSA 2013). The recommendation to group pesticides with common adverse outcomes together in common assessment groups and use dose addition to assess cumulative risk was viewed as a pragmatic and conservative default approach. The use of a default dose addition model, regardless of mechanism of action, was also recommended by an EFSA colloquium convened to harmonize of human and ecological risk assessment of combined exposure to multiple chemicals (EFSA 2015). This default approach was considered conservative and health-protective during "lower tier" assessments, and that the default approach could be modified to include more-than- or less-than-additive predictions if adequate data were available in "higher tier" analyses. However, the colloquium concluded that further development of available tools (e.g., PBPK models, quantitative structure-activity relationship models, adverse outcome pathways, etc.) are needed prior to routine integration of these models in human and ecological risk assessment.

C.16. EUROPEAN COMMISSION NON-FOOD SCIENTIFIC COMMITTEES

The European Commission non-food scientific committees (Scientific Committee on Consumer Safety, Scientific Committee on Health and Environmental Risks, Scientific Committee on Emerging and Newly Identified Health Risks) reviewed available scientific literature on the toxicity of chemical mixtures, drew conclusions from the review, and proposed a decision tree flow chart for evaluating the risk of chemical mixtures (EC 2012). Conclusions reached included the following:

1. "Chemicals with common modes of action will act jointly to produce combination effects that are larger than the effects of each mixture component applied singly. These effects can be described by dose/concentration addition.

2. For chemicals with different modes of action (independently acting), no robust evidence is available that exposure to a mixture of such substances is of health or environmental concern if the individual chemicals are present at or below their zero-effect levels.

3. Interactions (including antagonism, potentiation, and synergies) usually occur at medium or high dose levels (relative to the lowest effect levels). At low exposure levels, they are either unlikely to occur or are toxicologically insignificant.

4. In view of the almost infinite number of possible combinations of chemicals to which humans and environmental species are exposed, some form of initial filter to allow a focus on mixtures of potential concern is necessary. Several criteria for such screening are offered.

5. With regard to the assessment of chemical mixtures, a major knowledge gap at the present time is the lack of exposure information and the rather limited number of chemicals for which there is sufficient information on their mode of action. Currently, there is neither an agreed inventory of mode of actions, nor a defined set of criteria [on] how to characterize or predict mode of action for data-poor chemicals.

6. If no mode of action information is available, the dose/concentration addition method should be preferred over the independent action approach. Prediction of possible interaction requires expert judgement and hence needs to be considered on a case-by-case basis."

The decision tree flow chart for evaluating chemical mixtures, illustrated in Figure C-5, calls for:

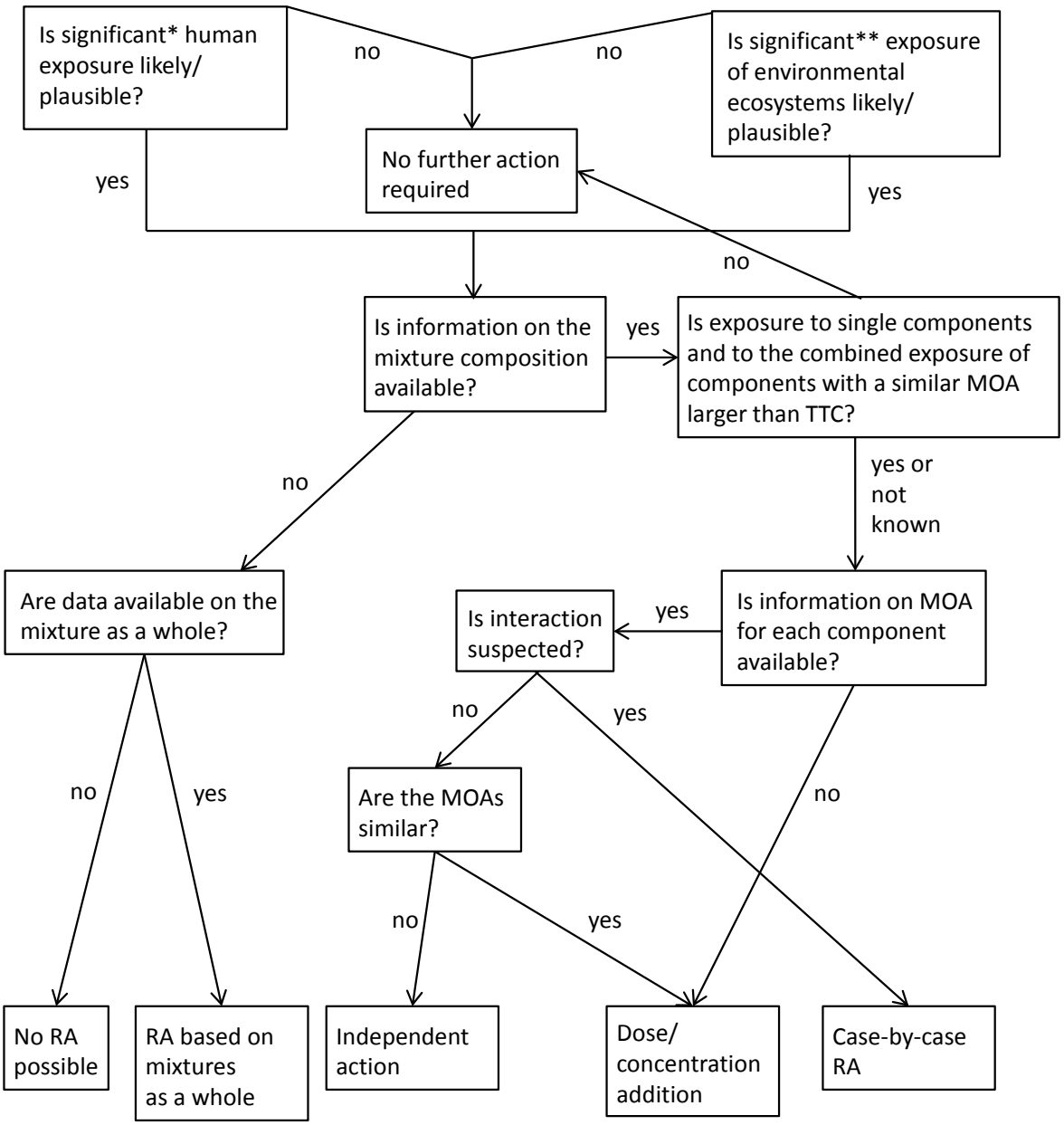
1. An initial assessment that significant human exposure is likely or plausible. Significance of exposure was to be determined by the frequency, duration, and magnitude of exposure.

2. Utilization of toxicity data on the mixture as a whole if available.

3. Use of dose-addition approaches if the mixture components produce common effects via a common MOA, and response addition approaches if mixture components are known to act independently.

4. Use of dose-addition approaches as a default approach if MOA information is not available.

Figure C-5. European Commission Non-food Scientific Committees' Recommended Decision Tree for Assessing Risks from Chemical Mixtures



*"Significant" exposure is determined by the frequency, duration, and magnitude of exposure.
**For the environment, an exposure-driven assessment without at least a preliminary risk characterization, as well as the TTC model, is hardly acceptable. Therefore, it must be considered as significant any exposure produced by emissions capable to modify the natural background conditions.
***Evidence for interaction can be found at various steps of the decision tree (e.g., comparing product information with compound-based assessment).

MOA = mode of action; RA = risk assessment; TTC = threshold of toxicological concern

Source: EC 2012 (© European Union, 1995–2015)

C.17. REFERENCES

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